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The Plant Disease Reporter is issued as a service to plant pathologists throughout the United States. It contains reports, summaries, observations, and comments submitted voluntarily by qualified observers. These reports often are in the form of suggestions, queries, and opinions, frequently purely tentative, offered for consideration or discussion rather than as matters of established fact. In accepting and publishing this material the Crops Research Division serves merely as an informational clearing house. It does not assume responsibility for the subject matter.

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The increase in the volume of pertinent material offered for publication in the Plant Disease Reporter has made it necessary to limit the subject matter and the length of articles accepted. The subject matter should emphasize new things in plant pathology, such as new records of disease occurrence, serious outbreaks and epidemics, conditions affecting development of plant diseases, techniques of investigation including instrumentation, new discoveries in control including new materials and their evaluation. Manuscripts will be limited to 12-double-spaced typed pages, including tables, graphs, and photographs. Because of reproduction costs photographs should be kept to a minimum. Insofar as possible, material should be presented as graphs rather than tables. Papers cannot be accepted for publication that report routine control experiments, reviews, bibliographies without annotation, results of routine surveys, mere summaries or lists of plant diseases. By following this procedure we hope to continue publishing all articles promptly.

Paul R. Miller

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OSMOTIC DESTRUCTION OF PLANT PARASITIC AND SAPROPHYTIC NEMATODES BY THE ADDITION OF SUGARS TO SOIL

William A. Feder[1]

Summary

Up to 100% of the nematodes were killed when sucrose or dextrose was added to nematode-infested soils at the rate of 1 to 5% by weight. As little as 1000 ppm of dextrose was nematocidal. The nematocidal action seemed to be caused by an increase in the osmotic value of the soil solution resulting from the addition of sugars. The nematodes living in the more concentrated soil solution were rapidly dehydrated and destroyed.

A routine search for various organic additives which would simultaneously cause an increase in the free-living saprophytic nematode populations of soil and the associated nematode-capturing fungi revealed that sucrose brought about a sharp reduction in the numbers of these nematodes. Twenty-four hours after 5% sucrose by weight was mixed with ordinary Florida citrus grove soil (Lakeland series), up to 100% of the nematodes originally present in the sample, including plant parasitic species, had been destroyed. The nematodes disappeared, leaving no microscopically visible traces in the liquid recovered from the funnels or from the surface of the Baermann screen (a "Scottie tissue"). Some 13 separate experiments on different available soil types with nematode populations which differed qualitatively as well as quantitatively repeatedly demonstrated the same effect (Table 1). Direct visual observations of the nematodes in sucrose-soil mixtures at 10-minute intervals after initial mixing revealed that some killing was evident 10 minutes after mixing took place. The observations seemed to rule out death by some pathogenic agent. This was confirmed by obtaining the same reaction when washed saprophytic nematodes were added to steam-sterilized soil to which 5% sucrose was then added. That sucrose itself was non-toxic to nematodes was demonstrated by the fact that saprophytic nematodes lived and multiplied in a 5% sucrose/water solution.

Table 1. Number of saprophytic and parasitic nematodes surviving 24 hours after the addition of 5% sucrose by weight to various soil samples. Soil types and moisture contents varied from sample to sample. Nematodes were extracted from 20-g soil samples by Baermann funnel.

Sample	Control	5% Sucrose
1	297	1
2	205	3
3	572	3
4	265	0
5	87	3
6	78	1
7	805	4
8	521	1
9	37	1
10	21	0
11	390	1
12	21	0
13	39	0

It is hypothesized that the addition of sucrose to a soil increases the osmotic value of the soil solution and that the nematodes, and perhaps other microorganisms living in the soil water films, are actually destroyed by dehydration through a process of exosmosis. The following experimental results are offered in support of this thesis.

Various plant parasitic as well as saprophytic nematodes were placed in dishes containing sucrose or glucose dissolved in distilled water. Concentrations ranged from 1 to 100% on a weight/volume basis. Approximate theoretical osmotic values of these molecular solu-

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Table 2. Percentage of plant parasitic and saprophytic nematodes killed after 1 hour's immersion in various concentrations of sucrose and dextrose solutions.

Concentration[a]	Sucrose: Panagrellus sp.	Sucrose: Pratylenchus brachyurus	Sucrose: Dolichodorus heterocephalus	Sucrose: Radopholus similis	Dextrose: Panagrellus sp.
1	0	-	-	-	0
3	0	-	-	-	23
5	0	-	-	-	44
7	18	-	-	-	84
10	30	20	21	47	100
20	83	71	32	85	100
30	97	100	90	100	100
40	100	100	100	100	100
50	100	100	100	100	100
60					
70					
75					
80					
85					
90					

[a]Concentration is expressed as percentage sugar on a weight/volume basis.

tions ranged from .67 atmosphere to 67 atmos. for sucrose and from 1.2 atmos. to 120 atmos. for glucose. One-hundred % of the nematodes died in 1 hour in the 30 to 40% concentration range of sucrose and in the 10% glucose solution. As noted by Jensen and Caveness (2), when nematodes were removed from the sugar solutions after 10 to 15 minutes and placed in tap water, many of them recovered their form and motility. However, longer periods of immersion in the sugar solutions caused irreparable damage and eventual death of the nematodes. These figures represent a theoretical osmotic value of about 22.4 atmos. for the sucrose and 12.3 atmos. for the glucose. This indicates that glucose has about double the effectiveness that could be predicted from its molecular weight and that there may be some other unexplained factor involved in the death of the nematodes (Table 2).

When a normal soil is allowed to dry out, the soil solution should become more concentrated. If sucrose or glucose is added to a slowly drying soil, the osmotic value of the soil solution should rise. This should, in turn, cause an increase in the percentage of nematodes which are killed. It should then be possible to draw a curve relating soil moisture with percentage mortality of nematodes in a drying 5% sucrose- or glucose-soil mixture. A number of such curves are shown in Figure 1. Soil containing a mixture of the plant parasitic awl nematode, Dolichodorus heterocephalus Cobb, and various saprophytic nematodes was thoroughly mixed and then spread out on a table to dry. Samples were removed from the pile periodically, always after thorough mixing. One-hundred-gram samples were mixed with sucrose or glucose at 1 and 5% by weight and 20 g of these samples were placed in the Baermann funnel

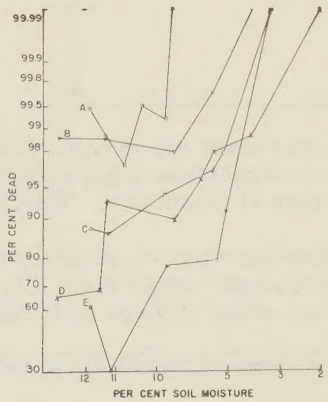

FIGURE 1. Relationship between percentage soil moisture and percentage nematodes killed 1 hour and 24 hours after mixing soils with either 1% or 5% dextrose or sucrose. Soils were oven-dried and the percentage soil moisture calculated in terms of 100 g wet weight.

1 hour and 24 hours after the initial carbohydrate-soil mixtures were made. Another 100-gram sample was taken at the same time and oven-dried to ascertain the moisture content. Curves C and D in Figure 1 compare 5% glucose and sucrose respectively 1 hour after mixing. Curve E shows the effect of 1% glucose 1 hour after mixing. Curves A and B compare 5% glucose and sucrose respectively 24 hours after mixing. These curves indicate that there is a positive relationship between a diminishing soil moisture content and an increase in nematode mortality in a soil-sucrose or glucose mixture. It is highly probable that as the soil-moisture content decreases, the concentration of dissolved carbohydrate increases with a concomitant increase in the osmotic value of the soil solution. Since the level of the nematode population in the untreated soils remained essentially unchanged as soil moisture dropped from 12.6% to below 3%, the increase in nematode mortality in the treated soils is probably due to the adverse effects of the steadily rising osmotic value of the soil solution brought about by the increasing carbohydrate concentration in that solution.

This evidence indicates that sucrose or glucose mixed with the soil is highly effective in reducing populations of both parasitic and saprophytic soil-dwelling nematodes.

The data also seem to support the theory that these nematocidal effects are brought about by an alteration in the osmotic value of the soil solution in which these nematodes live. The quantitative addition of these carbohydrates to nematode-infested soils of known moisture content gives predictable percentages of nematode kill. Preliminary studies indicate that these effects can be readily repeated under greenhouse and field conditions. A full report of these and other field studies will appear later.

This is apparently the first report of a harmless, non-toxic chemical being used as a nematocide, although some evidence of this property of dextrose was noted by Dropkin, et al. (1). It also represents the first time that variations in the osmotic value of the soil solution have been controlled for killing microorganisms in these solutions. Early results indicate that dextrose mixed in the soil is nematocidally effective in small enough amounts (1000 ppm) to make the process practical and economically feasible. Quite as important, however, should be the recognition that a new approach to the study of what may constitute nematocidal activity has been presented. Simple non-nematoxic chemicals can be used to alter the osmotic values of the soil solution in a controlled, quantitative fashion, thus destroying soil-dwelling nematodes. Other chemical compounds which can cause these effects have not been investigated. There may be many such compounds that are cheaper and more effective than dextrose. A new field of chemical exploration has been opened to investigation. Further, dextrose as a nematocide in the hands of the average farmer of the world would be a safe, harmless, simple material which could be applied easily and at a minimum risk to human beings or animals and it is harmless to the plants it protects.

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CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, ORLANDO, FLORIDA

LOCAL DISSEMINATION OF INTERNAL CORK VIRUS OF SWEETPOTATOES

Dale H. Habeck, L. W. Nielsen, and Charles H. Brett[1]

Summary

Local dissemination of internal cork virus of sweetpotatoes was studied in North Carolina in 1957 and 1958. In 1958 the data indicated that virus dissemination occurred during late July. The magnitude of local dissemination was much higher in 1957 than in 1958 and was associated with larger populations of potential vectors. The infection data clearly indicate that virus dissemination is inversely related to the distance from the source of inoculum, although this may be influenced by the topography of the intervening terrain.

Following the discovery of the internal cork virus (ICV) of sweetpotatoes in South Carolina in 1944, the disease was soon recognized in most of the sweetpotato-growing areas of the country. This regional dissemination evidently resulted from movement of infected propagating roots and plants from one growing area to another.

Various observations and studies (1, 2, 5, 8, 9) indicate that insects are responsible for the local dissemination of ICV. Nusbaum (9) reported that supposedly virus-free stocks became infected in the field and that the percentage of infected plants increased when the same stock was propagated year after year. Nielsen (5) reported that uncaged sweetpotato plants located near infected plants in the greenhouse became infected while caged plants were not infected. Nielsen and Person (8) stated that the ICV spreads to healthy plants, if a source of inoculum is nearby. Virus spread occurred in widely separated areas of North Carolina, indicating that a vector was present in these areas. Over a 5-year period following the discovery of the disease (6), the percentage of infected roots from commercial plantings in North Carolina increased as follows: 4% in 1947; 12.9% in 1948; and 71.2-90% in 1952.

Studies on the local dissemination of the virus from infected to healthy plants indicate that distance between infected and virus-free plants is an important factor in determining the number of healthy plants that become infected. Martin and Kantack (3, 4) reported that roots from virus-free plants grown adjacent to an infected planting were more heavily infected than roots from cork-free plants grown 100 or more yards from infected plants. The infection of virus-free plants was inversely related to the distance between the healthy and infected plants. At a distance of 10 feet there was nearly 20% infection, while at 160 feet there was 2.3% infection. Sweetpotatoes grown on an isolated coastal island in North Carolina for several years remained free of the disease (7).

There were three objectives in this investigation: 1) to determine the time of virus transmission during the growing season, 2) the seasonal dissemination of ICV within a planting, and 3) the distance of virus dissemination from a given source of inoculum.

METHODS AND MATERIALS

In all experiments, virus-free plants of the Porto Rico variety were used to estimate virus movement from a source of inoculum. The virus-free plants were planted either adjacent to or at various distances from infected plants, depending on the objective of the experiment. Details on the disposition of virus-free plants with respect to the infected plants will be given in the appropriate results section. Sweetpotato sprouts were planted between June 12 - 30 and the rows were fertilized prior to planting with approximately 1000 pounds/acre of 2-12-12 placed in bands. The potatoes were harvested between October 15 and November 12. Harvested roots were stored 5 to 6 months at 75° F to intensify symptom expression. Disease diagnosis was based upon the presence of internal necrosis in cross-sectional root slices about 1/16-inch thick.

Experimental plantings were made at two locations: in the vicinity of Clayton, N. C., where commercial production of sweetpotatoes is light, and at Benson, N. C., a heavy sweetpotato-producing area.

Insect surveys were made at regular intervals throughout the growing season at both locations with a standard insect sweep net during 1957 and 1958. Leafhoppers were identified to

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species. In 1958 additional surveys were made for aphids by checking 20 and 10 plants per plot for the first and second counts, respectively, and 200 leaves per plot thereafter at irregular intervals throughout the season.

RESULTS

Time of Virus Transmission: Virus-free plants were set at different dates adjacent to infected potatoes, for the purpose of determining the time of vector flight (virus dissemination) during the early growing season. Fifty virus-free plants were set June 12, 1958 in a row parallel to a field of infected potatoes (set June 3) and 7 feet from them. Similar plantings were made at successive 2-week intervals until four plantings were established. To provide infected roots of comparable development, 50 infected plants were also set on each date in the same field.

Data in Table 1 show that the virus spread to the disease-free plants. Roots from comparable virus-free plants grown in isolation had no symptoms. About the same percentage of roots (10.6 to 13.2) from plants set June 12, 26 and July 12 had internal necrosis. The percentage of roots with symptoms from the fourth planting (July 27) was considerably less. These data are interpreted as indicating that the vectors appeared after the July 12 planting and remained active for a short time or in diminishing numbers after the July 27 planting. This interpretation is based on the assumptions that: 1) the inoculum was continuously available, 2) the vector(s) acquires the virus and disseminates it at random, and 3) the Porto Rico sweetpotato plants are equally susceptible regardless of age.

Table 1. Effect of planting time on incidence of internal cork symptoms in roots after warm temperature storage.

	Virus-infected plants		Virus-free plants	
Planting date	Number roots harvested	% roots with cork	Number roots harvested	% roots with cork
June 12	243	19.3[a]	363	13.2
June 26	301	69.4	436	10.6
July 12	204	59.8	410	11.5
July 27	--[b]	--	233[c]	2.6

[a]Probably planted virus-free plants by mistake.
[b]Plants from field plant bed, and jointly infected with Fusarium oxysporum f. batatas. Nearly all plants died during growing season.
[c]Largest roots about 1 1/2 inches in diameter.

The percentage of roots from infected plants with internal cork symptoms was much greater for two dates of planting. The low reading for roots from the June 12 planting (19.3%) is very similar to the values for roots from the virus-free plants, and indicates that virus-free plants were erroneously set in this plot. Several weeks after setting virus-infected plants on July 27, it was evident that the plants were also infected with Fusarium oxysporum f. batatas. Only a few Fusarium infected plants survived the growing season, and these produced no usable roots.

Virus Dissemination Within a Planting: During the 2 years of these studies, the seasonal dissemination of ICV within a planting of virus-free and diseased plants was measured. Each year a planting consisting of four rows 50 feet long was established 1500 or more feet from the nearest garden or experimental plantings of sweetpotatoes. Each planting contained 180 virus-free plants and 20 infected plants. The plants were spaced at approximately 1-foot intervals in the rows and every tenth plant was virus infected. Prior to harvest, the infected plants and their roots were removed manually and discarded. The roots from the originally virus-free plants were examined for internal cork symptoms after warm temperature storage.

There was dissemination of ICV within the plots both years. However, the magnitude of dissemination, as reflected by percentage roots with internal necrosis, was very different for the two growing seasons -- 41.3% in 1957 and 0.2% in 1958. This large difference in ICV dissemination for the two growing seasons was also evident in other experiments conducted during the same time, as will be pointed out in the following section.

It is postulated that the difference in ICV dissemination for the 2 years was associated with the relative abundance of vectors during the two growing seasons. Although the vector or vec-

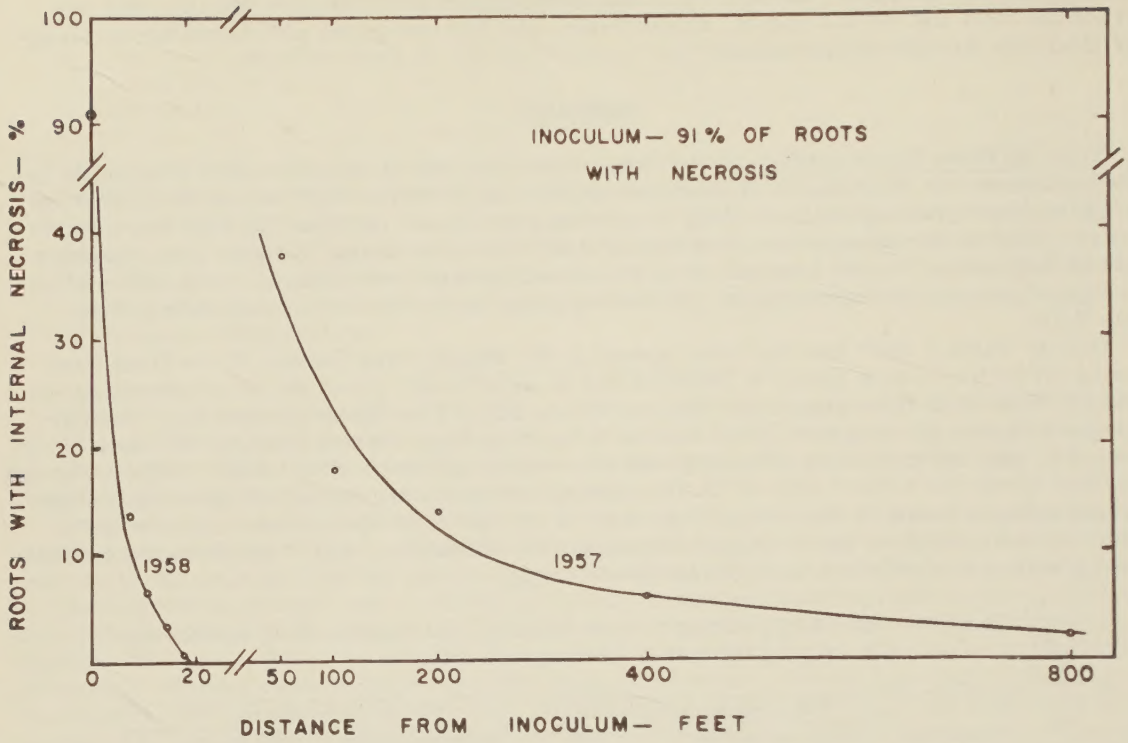

FIGURE 1. Local dissemination of internal cork virus away from a source of inoculum, Benson-1957, Clayton-1958.

Table 2. Dissemination of internal cork virus to virus-free potatoes planted in two directions from an infected crop, Clayton, 1958.

Location of virus-free plants relative to infected crop[a]		Number of roots	% roots with cork symptoms
Direction	Distance (feet)		
Northeast	50	200	3.5
	100	200	5.0
	200	200	0.5
	400	160	0.0
	1200	200	3.5
Southwest	50	200	7.0
	100	160	0.0
	400	109	0.9
	800	200	1.5

[a]90% of harvested roots from commercial crop had symptoms.

tors of ICV in North Carolina have not been determined, they are probably homopterous insects. A comparison of leafhoppers and aphids collected with a sweep net during the two growing seasons clearly shows that both groups were more numerous in 1957 than in 1958. At the Clayton location aphids were three times more abundant in 1957 than in 1958. The same was true for 13 species of leafhoppers including Aceratagallia sanguinolenta, Carneocephala flaviceps, Empoasca abrupta, E. fabae, Macrosteles fascifrons, Exitianus exitiosus, Deltocephalus sonorus, and others.

Distance of Virus Dissemination from Infected Sweetpotatoes: The extent of virus movement away from a commercial field containing high percentages of infected plants was studied by planting virus-free plants at various distances from commercial sweetpotato plantings. In

1957 virus-free plants were set in duplicate 25-foot plots at 50, 100, 200, 400, and 800 feet in a southeastern direction from a field of cork-infected sweetpotatoes located in eastern Harnett County. The paired plots, each containing 25 hills of sweetpotatoes, were parallel with the field border and 25 feet from each other and extended through cotton and soybean fields. A second test was conducted in 1958 near Clayton, North Carolina. Paired 25-foot plots of virus-free plants were planted in a southwest direction at 50, 100, 400, and 800 feet from the infected potatoes. Virus-free plants were also set in a northeast direction at 50, 100, 200, 400, and 1200 feet from the infected crop. Each year a bushel of potatoes from the commercial crop was collected at harvest to determine the inoculum potential within the crop.

The infection data from the 1957 experiment in Harnett County clearly indicate that virus dissemination is inversely related to the distance from the infected crop (Fig. 1, 1957). At a distance of 50 feet, 38% of the roots had symptoms. As the distance between the infected and virus-free plants increased, the percentage of roots with symptoms decreased, and 1.5% of the roots grown at a distance of 800 feet had internal cork symptoms.

Variable data were obtained from the 1958 test located near Clayton. The virus spread to the northeast 1200 feet and to the southwest 800 feet (Table 2). There was some evidence of the inverse relationship for a distance of 400 feet in both directions, but at greater distances this relationship did not hold. Roots with symptoms were not found in the plots located 400 feet to the northeast and 100 feet to the southwest of the infected crop, yet roots with symptoms were found in plots at greater distances in both directions. The failure to get infection in these plots may have been associated with the topography of the land. The infected crop was located on the summit of a hill about 30 to 70 feet higher than ravines in either direction and the plots with little or no infection were located on the receding slopes or in the ravine in either direction from the summit. The most distant plots in both directions were on land increasing in elevation and approaching that of the infected crop.

In these studies the magnitude of virus dissemination in 1958 was much less than in 1957. As already explained, this reduction in dissemination in 1958 was associated with smaller populations of potential vectors. The failure to obtain infection in some of the plots may also be associated with a sparse vector population.

The distances over which the ICV was disseminated in the experiments, and the location of test plots in other crop plants, suggest that the vector is fairly mobile. However, data were also obtained from another experiment which indicates the vector is rather immobile.

Dissemination over a short distance was detected by planting virus-free sweetpotatoes adjacent to an infected planting. Virus-free plants were planted in four parallel rows (3 1/2 feet wide) adjacent to an infected planting in 1958 at the Clayton Experiment Station. A distance of 7 feet was left between the infected plants and the virus-free plants. The roots from each row of virus-free plants were examined separately for root symptoms. When the infection data were plotted against distance from the edge of the infected planting, an inverse relationship was again evident (Fig. 1, 1958). Although the percentage of cork for the entire plot was only 7.2, there was a negative correlation between disease incidence and the short distance of 17 feet. This limited spread suggests that a slowly mobile insect (wingless aphids or other nymphal forms) transmitted the virus in these plots.

DISCUSSION

ICV dissemination in North Carolina is presumed to be dependent on a vector or vectors. Various aphids have been reported as vectors of the virus (1, 2, 10). In 1958, a season of relatively low virus dissemination in North Carolina, the data indicated that virus dissemination occurred in late July. However, for growing seasons such as 1957, when potential insect vectors were much more numerous, virus dissemination may have occurred earlier, later, or over a longer period of time. The relative abundance of insect vectors is probably related to climatic and biologic factors such as winter or summer temperatures, rainfall, predators and parasites.

The inverse relationship between distance from a source of ICV inoculum and virus dissemination is in general agreement with the results of Martin and Kantack (3, 4). In our studies there was greater infection (38% at 50 feet, Fig. 1) over longer distances (1200 feet, Table 2). These data have important bearing on the production of potatoes free of ICV. Martin and Kantack (4) reported that little transmission occurred in Louisiana if virus-free plants were located 100 yards from an infected crop. Under North Carolina conditions this distance may be adequate for table stock production, if virus-free seedstock is used each year, but is inadequate for the production of virus-free seedstock. A minimum safe distance at which virus-

free plantings must be separated from infected plantings is difficult to establish. Distances which might be quite safe under usual conditions could prove completely inadequate during strong winds associated with hurricanes. Under these conditions, viruliferous insects might be carried long distances.

In addition to the distance between virus-infected and noninfected plants, land topography and the type of vegetation on the intervening terrain may be important factors. The intensity of sweetpotato production (inoculum potential) in an area may also be an important factor in determining the effectiveness of isolation in maintaining and producing virus-free propagating stock.

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HYMENOMYCETOUS FUNGI EXCEPT POLYPORUS ASSOCIATED WITH WOOD DECAY OF LIVING PEACH TREES IN SOUTH CAROLINA[1]

D. H. Petersen[2]

Abstract

Twenty-two species of Hymenomycetous fungi excluding Polyporus spp. were found associated with decayed woody tissues of living peach trees in South Carolina. Of these species, Armillaria mellea, Clitocybe tabescens, and Stereum complicatum were found most frequently. Sixteen species are reported apparently for the first time on peach in the United States.

INTRODUCTION

The short life of peach trees is a major economic problem of orchardists of the southeastern United States. One cause of the rapid decline of many trees appears to be the decay of heartwood and sapwood by various species of Hymenomycetes. These wood-rotting fungi are active much of the year because of the moderate temperatures and frequent high humidities characteristic of this region. A knowledge of the fungous species associated with peach wood decay is an essential first step in establishing causal relation to tree decline.

No intensive survey of the Hymenomycetous species associated with decay of woody tissues of living peach trees has been reported and the literature is particularly barren of species reported from the Southeast. Agriculture Handbook No. 165 (9) lists 13 species of Hymenomycetes excluding Polyporus spp. as causal agents of diseases and wood decay of peach in the United States. Two were listed as cosmopolitan and might be expected to occur in the Southeast: Armillaria mellea and Schizophyllum commune. From States in the Southeast were listed Clitocybe tabescens and Fomes pinicola. Rhoads (7) reported Clitocybe tabescens as occurring in Georgia and South Carolina.

This paper reports the results of a survey of the species of Hymenomycetes excluding Polyporus spp. which were found associated with wood decay of living peach trees in South Carolina[3].

PROCEDURE

During 1955 to 1960, collections of Hymenomycetous sporophores were made from living trees in over 100 orchards in South Carolina. Six orchards in various geographical areas of the State were selected for periodic surveys each year. The other orchards, surveyed at random during the various seasons of a year, included plantings of all age groups, on many soil and site-location types, and on original and replanted sites.

In surveying an orchard, the procedure used was to walk the center of a strip 4-trees wide. When fruiting bodies were seen, they were examined for tentative identification. The fruiting body or a group of them and a section of decayed host tissue were removed if the fungus could not be identified readily, if it was a species or form not collected previously, or if its location on the host was unusual.

RESULTS AND DISCUSSION

Twenty-two species of Hymenomycetes other than Polyporus spp. were found associated with decaying tissue of living peach trees in South Carolina. A brief discussion of each species follows. Table 1 indicates, in a generalized manner, the population frequency of the various species. The omission of literature citations in the discussion indicates the species was not found reported on peach in the United States.

Agaricaceae: Armillaria mellea (Vahl ex Fr.) Quél. was found frequently only in the

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[1]Cooperative investigations of the Crops Research Division, Agricultural Research Service, United States Department of Agriculture and the South Carolina Agricultural Experiment Station. Technical Contribution No. 345, South Carolina Agricultural Experiment Station, Clemson.

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[3]Acknowledgment is given to J. L. Lowe, New York State College of Forestry, Syracuse, New York; R. W. Davidson, Forest Service, Beltsville Forest Disease Laboratory; J. A. Stevenson and P. L. Lentz, Crops Research Division, Agricultural Research Service, Beltsville, Maryland, for aid in identification.

Table 1. Frequency of occurrence of Hymenomycetes on living peach trees in South Carolina.

Species	Frequency of occurrence[a]	Species	Frequency of occurrence[a]
Armillaria mellea	4	Merulius confluens	2
Clitocybe tabescens	4	Poria ambigua	3
Panus rudis	3	Poria corticola	1
Panus stypticus	1	Odontia spathulata	1
Schizophyllum commune	3	Steccherinum ochraceum	1
Fomes applanatus	1	Laxitextum bicolor	1
Fomes lobatus	1	Laxitextum crassum	3
Fomes subroseus	1	Peniophora affinis	1
Lenzites betulina	1	Stereum albobadium	2
Lenzites saepiaria	1	Stereum complicatum	5
Lenzites trabea	1	Stereum ochraceo-flavum	1

[a]Symbols for the numbers of times found: less than 10 -- 1; 10-25 -- 2; 26-100 -- 3; 101-1000 -- 4; over 1000 -- 5.

deepsand area of the northcentral part of the State. Fruiting bodies were abundant at the bases of infected trees during rainy periods in October and November. Mycelium was associated with a soft, somewhat stringy, white rot of the roots and crowns. The pathogenic relation of A. mellea to stone-fruit trees is well established and the subject of recent reviews by Clayton (1) and Groves (5).

Clitocybe tabescens (Scop. ex Fr.) Bres. was found to be a common species on roots and crowns of dead or declining peach trees. As fruiting bodies were rarely seen, identification of the species was often based on the characteristics of the decay, mycelium, and growth in culture. C. tabescens can be separated from similar species, such as A. mellea (6). Invasion of underground parts of peach trees by the mycelium of Polyporus versicolor often results in the production of mycelial mats and decayed tissues like those of C. tabescens (6). Unless sporophore production can be induced by extricating the infested tissues from the soil, the characteristics of growth in culture were found to be the only reliable means of separating these two species. Rhoads (6) described the cultural characteristics of C. tabescens and Davidson, et al. (2) those of P. versicolor. Savage, et al. (8) reported the occurrence of C. tabescens in Georgia and South Carolina.

Panus rudis Fr., while not abundant, was seen frequently on living peach trees. Most of the sporophores were found on limbs apparently damaged by sun-scald, although they were noted infrequently on dead trunk tissue or on large pruning cuts. They were associated with a limited area of decay in which the woody tissues were soft and white to straw-colored.

Panus stypticus Fr. was rarely found on peach in South Carolina. Sporophores were noted on small pruning cuts less than 1 1/2 inches in diameter and on dead tissue of living limbs. The area of the associated decay was very limited and the decayed tissues were soft, somewhat punky, and light straw-colored.

Schizophyllum commune Fr. was common on peach trees throughout South Carolina. Sporophores were seen most often on dead trees. On dead tissue of living trees, they were associated with a firm, straw-colored rot. The ability of S. commune to cause decay in peach wood has not been clearly established (3, 4).

Polyporaceae (except Polyporus): Fomes applanatus (Pers. ex S. F. Gray) Gill. was collected rarely. The sporophores, found at the base of living trunks, were associated with extensive decay, mostly in the heartwood. Tissues in the early stages of decay were mottled white and cracked into somewhat rectangular pieces, whereas in the more advanced stages of decay, they appeared uniformly white, soft, and spongy. The fungus was reported on peach in Connecticut and New Jersey (9) and as parasitic on prunes in Oregon (10).

Fomes lobatus (Schw.) Cke., found only once during the survey, was fruiting on a large pruning cut made in the crotch area of the tree. The associated white, soft decay was extensive in the crotch area and the tree was in an advanced state of decline.

Fomes subroseus (Weir) Overh. was found infrequently on dead limbs of living trees. The sporophores were associated with an extensive, brown, cubical rot. Zeller (11) reported the fungus, which he termed Trametes subrosea, as a predominant heart rot of peach trees in California, Oregon, and Washington.

Lenzites betulina (L. ex Fr.) Fr., L. saepiaria (Wulf. ex Fr.) Fr., and L. trabea Pers. ex Fr. were rare on peach in South Carolina. Most sporophores were seen on dead tissues of living limbs. The decayed tissues associated with L. betulina were soft to punky and light

straw-colored. The decay associated with the fruiting bodies of L. saepiaria and L. trabea was brown and checked. L. saepiaria was reported as a serious wound parasite on peach in Oregon (10).

Merulius confluens Schw. was seen occasionally on living trees. Its sporophores, found on dead tissue of living limbs and trunks, were associated with an extensive, soft, light straw-colored rot.

Poria ambigua Bres. was seen rather frequently during the fall. The sporophores were found on the basal portions of dead limbs of living trees or on dead tissues of living limbs and trunks. Occasionally they were noted over-running the soil in the immediate vicinity of a tree trunk. The sporophores were associated with a white rot of the sapwood.

Poria corticola (Fr.) Cke. was rarely seen. The fruiting bodies occurred on dead tissues of living limbs and trunks and were associated with a soft, white rot.

Hydnaceae: Odontia spathulata (Fr.) Litsch. appeared to be rare on peach in South Carolina. Its sporophores were noted at the soil line on necrotic tissues of living tree trunks and were associated with a very restricted, firm, brown rot.

Steccherinum ochraceum (Fr.) S. F. Gray was seen rarely. Fruiting bodies occurred on dead trunk tissue from the soil line to the crotch area of living trees. They were associated with an extensive sapwood rot in which the tissues become soft, punky, and straw-colored.

Thelephoraceae: Laxitextum bicolor (Pers. ex Fr.) Lentz was seen rarely during the survey. The sporophores, found on dead tissue of living limbs, were associated with a restricted brown rot of the sapwood and heartwood.

Laxitextum crassum (Lév.) Lentz was found rather commonly. Its fruiting bodies were seen most frequently on dead tissue of living scaffold limbs or on dead limbs of living trees. They were associated with a soft, white rot of the sapwood and heartwood.

Peniophora affinis Burt was identified but once. The sporophores were on dead tissue of a living tree trunk. The characteristics of the limited decay were not determined.

Stereum albobadium (Schw. ex Fr.) Fr. was seen fruiting rather commonly on dead peach twigs on the tree and on the ground. One collection was made from a pruning cut, but the characteristics of the limited decay were not determined.

Stereum complicatum (Fr.) Fr. was the most commonly found Hymenomycete on peach trees in South Carolina. While it was seen fruiting on all above-ground woody tissues, pruning cuts served as the most frequent site. During mid-summer 10 to 15 or more clusters of sporophores were often seen on as many pruning cuts per tree. They were associated with a white rot, primarily of the sapwood.

Stereum ochraceo-flavum (Schw.) Ell., seen but once, was found fruiting on a pruning cut of a living limb. The characteristics of the limited decay were not determined.

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OCCURRENCE OF SNOW MOLD, TYPHULA ITOANA IMAI, ON WINTER OATS IN PENNSYLVANIA[1]

H. G. Marshall[2] and Richard D. Schein[3]

As the adaptation zone of fall-seeded oats is pushed farther north through selection and breeding for winter-hardiness, the plants are subjected to disease stresses not previously encountered. In the one available reference to snow mold damage to winter oats, the organism involved was Fusarium nivale (Fr.) Ces.[4]. This paper reports severe killing of winter oats by another snow mold, Typhula itoana Imai, in areas of a breeding nursery located in Clearfield County, Pennsylvania, during 1959-60.

The nursery was covered with a heavy snow accumulation continuously from February 15 to March 31. Snowfall from three storms during this period was over 60 inches. Dead oat plants matted tightly to the ground and with a whitish-gray, bleached appearance were prominent when survival notes were taken on April 25 (Fig. 1). Close examination of the culms and leaves revealed a covering of dense mycelial growth and numerous reddish-brown sclerotia. The killing caused by the fungus in one area of an advanced-generation yield test (Fig. 2) is shown in Table 1. As little as 35% survival of certain lines planted nearby in single rod rows was recorded.

Table 1. Survival of winter oat varieties and lines in an advanced-generation rod-row yield trial in Clearfield County, Pennsylvania, during 1959-60.

C. I. No.	Kind	% survival[a]
7480	Wintok Selection x Hairy Culberson	90
7502	Wintok Selection x Hairy Culberson	80
6571	Bronco	80
5107	LeConte	80
7132	(Trav. x (RR x V-R)) x (Fulwin-Wintok)	80
6904	(Lee - Victoria) x Forkedeer	80
7129	Wintok x Atlantic	79
7500	Wintok Selection x Hairy Culberson	75
7482	Hairy Culberson x Nysel	74
6980	Pentagon Reselection ("Ballard"): Ky. 45-65	74
7503	Hairy Culberson x Nysel	72
6903	Norline	70
7499	Wintok Selection x Hairy Culberson	68
3424	Wintok	50
7504	Hairy Culberson x Nysel	50
6572	Dubois	47

[a]Calculated from estimated fall and spring stands.

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[4]Haenseler, C. M. 1948. Varietal susceptibility of winter oats to snow mold (Fusarium nivale) in New Jersey. Plant Disease Reptr. 32:175-176.

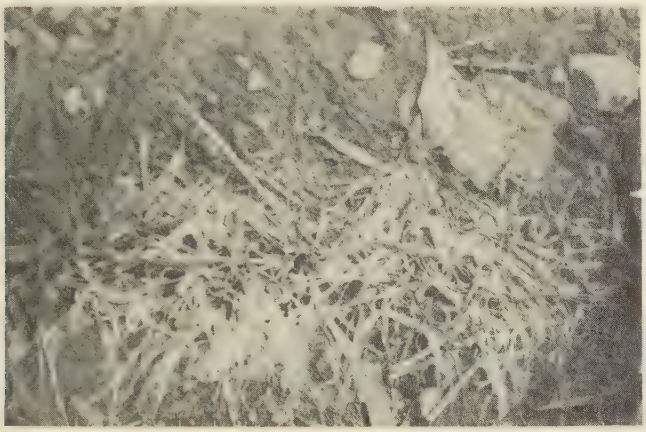

FIGURE 1. Portion of a row of winter oats showing typical appearance of dead plants infected with Typhula itoana.

FIGURE 2. Area of winter oat nursery indicating the extent of killing caused by Typhula itoana.

The primary objective of the winter oat breeding program in Pennsylvania is to develop varieties with greater winter-hardiness than presently available. Such varieties should result in a northward movement of winter oat production, and should this occur snow mold conceivably may become an important cause of winterkilling in some areas in certain years.

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FORECASTING POTATO LATE BLIGHT IN WESTERN NEBRASKA[1]

Jack R. Wallin and Max L. Schuster[2]

Summary

The use of temperature-relative humidity data in forecasting late blight was tested during an 8-year period, 1952-1959 inclusive, in western Nebraska. The data were obtained from weekly recording hygrothermographs in potato fields at the Scottsbluff Experiment Station from 1952-1959 inclusive; Alliance from 1954-1958 inclusive; bottom land at Scottsbluff from 1954-1957 and 1959; Mitchell from 1957-1959; and Hay Springs from 1954-1959.

The late blight fungus developed or did not develop as predicted in all years but 1953, when forecasts were interrupted in August. In all years but 1953, growers were warned of late blight development before its detection in potato fields.

During the 8-year period, 1959 was the only year in which late blight was not reported from western Nebraska.

Prior to 1951 the late blight fungus, Phytophthora infestans (Mont.) d By., caused only occasional losses in the irrigated highlands of western Nebraska. In fact, the disease was unique in that tuber symptoms, but not foliage symptoms, were noted. In 1951 a late blight epiphytotic occurred in potatoes around Alliance and Scottsbluff.

Irrigation and rainfall provide the moisture for potato culture in western Nebraska. The average annual rainfall for the area is 13.8 inches and 7.06 inches for the 4-month period June through September. The rainfall for the 4-month period for four stations in western Nebraska is shown in Table 1. Such a nominal amount of rainfall must be supplemented by irrigation to support potatoes.

Table 1. Average inches of rainfall for four stations in western Nebraska for June through September, 1906-1959[a].

Location	June	July	August	September	Total
Alliance	2.81	1.72	1.61	1.34	7.48
Hay Springs	3.15	2.10	1.68	1.57	8.50
Mitchell	2.99	1.30	1.21	1.18	6.68
Scottsbluff	2.78	1.45	1.22	1.29	6.74
Mean	2.93	1.64	1.43	1.34	7.35

[a]Computed from U. S. Weather Bureau Climatological Data.

The low rainfall offered an ideal situation for late blight forecasting on the basis of temperature and relative humidity criteria that have been used successfully in other north-central States (6, 7, 8, 9, 10). In the northeastern States rainfall in various combinations with temperature has been used as a criterion for late blight forecasting (1, 2, 3, 4, 5). In the northeast certain rainfall values coincident with given temperature limits have been translated into favorable blight conditions. However, the sparse western Nebraska rainfall is probably an unreliable indicator of the numerous periods when free water is present on the foliage, allowing the pathogen's secondary infection processes to occur. Hence, other criteria must be found.

Water supplied through sub-irrigation would be expected to promote growth of the host but not the fungus because sub-irrigation would not provide the free water on the foliage essential to the pathogen's secondary infection processes (spore germination and subsequent germ tube penetration). However, irrigation water probably increases the relative humidity in the canopy

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of potato foliage and supplies the moisture utilized in dew and fog formation, and, therefore, indirectly provides the relative humidities of 90% or more essential to sporulation and the moisture for dew deposition essential to spore germination. These conditions can be recorded by a hygrothermograph located among the plants.

The irrigated potato fields offered the environment for testing the use of the hygrothermograph in forecasting potato late blight under low rainfall conditions. Sheltered hygrothermographs placed among the plants recorded the prevailing temperatures and humidities in the foliage. In turn, these records were analyzed to estimate the number of secondary-infection periods according to the criteria used to predict the development of the pathogen.

Eight years' results with temperature-relative humidity criteria in forecasting potato late blight for the western Nebraska potato region are presented herein.

MATERIALS AND METHODS

Sheltered, 7-day recording hygrothermographs were placed in potato fields at Scottsbluff, Alliance, Mitchell and Hay Springs. The louvered shelters were placed about 12 to 15 inches above the soil so that the foliage grew up around the shelter. The U. S. Weather Bureau temperature-relative humidity readings taken 6 feet above ground do not reflect the true climate in the potatoes. Volunteer observers changed the charts weekly and mailed them to Ames, Iowa for analyses.

At least 10 hours of a mean temperature in the 60° to 80° F range coincident with relative humidities ≥ 90%, 13 hours of a mean temperature in the 54° to 59° range, or 16 hours of a mean temperature in the 45° to 53° range were considered minima for successful completion of the secondary infection cycle. The greater frequency and longer duration of these "favorable" periods were expected to result in more sporulation and secondary infection. These time and temperature ranges were extrapolated in an experimental table of late blight severity values or estimates[3]. One period of value of 1 per week was considered sufficient to promote at least one generation of the pathogen, and a value of 3 per week was considered sufficient to stimulate enough secondary infection to warrant fungicide application. The extent of the pathogen's weekly development was predicted from the number and total value of favorable periods. The pathogen was considered dead after three consecutive weeks without a favorable period.

In the absence of relevant information concerning inoculum and control programs, the presence of the pathogen was assumed in the seed stocks and the forecasts applied to unsprayed fields of susceptible plants. However, frequent field observations for late blight conducted during the season to establish the presence or absence of inoculum provided the forecaster a current account of the actual blight incidence in a locality, thus informing him of the accuracy of the predictions.

RESULTS

The forecast results are summarized in Table 2.

In 1952 only the Scottsbluff weather-late blight station was operating in western Nebraska. During the week ending August 10 the only three favorable periods were recorded. As shown in Figure 1, there were six favorable periods in the 3 months June 1-September 1. During this time, there were no favorable periods for 28 days. Hence, late blight was not predicted in the Scottsbluff area. A trace was found about mid-September but the disease failed to spread. Hence, no blight warnings were issued and none were needed.

In 1953 three weather-late blight stations (Scottsbluff Experiment Station, Hay Springs, and Alliance) were operating. The forecasts were abreast of the situation until the week ending July 26. The blight severity values data shown in Figure 1 indicated that up to July 26 some blight development could be expected at Mirage Flats near Hay Springs, Nebraska and blight was found August 25. The disease was found at Alliance on August 20 and at Scottsbluff-1 and -2 on August 25. After August 25, the forecasts reliably predicted the further development of the fungus in the local areas surrounding the hygrothermographs.

The forecast issued September 4 suggested continued blight development from lesions in which the fungus survived the heat during the week ending August 30. The temperature maxima were above 90° F for 7 days. Apparently the pathogen survived in some lesions because heavy damage was noted in September in some low fields at Scottsbluff and in irrigated fields at Alliance. The favorable periods during August resulted in slight blight development during September in fields containing the instruments.

3Wallin, Jack R. 1957. Plant Disease Situation Report 28. U. S. Dept. Agr., Mimeo.

Table 2. Summary of late blight forecasts and late blight incidence in the western Nebraska potato area during the 8-year period 1952-1959, inclusive.

Year	Locality	Date first blight forecast issued	Date blight first reported	Final blight severity in general area
1952	Scottsbluff - 1[a]	None	Sept. 15	0 - trace[b]
1953	Alliance	July 28	Aug. 20	Severe
	Hay Springs	July 28	Aug. 15	Trace
	Scottsbluff - 1	July 28	Aug. 25	Trace-severe
1954	Scottsbluff - 2[c]	Aug. 17	Aug. 23	Severe
1955	Scottsbluff - 2	June 28	Aug. 17	Moderate
1956	Scottsbluff - 2	Aug. 11	None	None
1957	Alliance	July 29	Aug. 3	Severe
	Hay Springs	July 29	None	None
	Mitchell	July 29	Aug. 3	Trace-severe
	Scottsbluff - 1	July 29	Aug. 3	Severe
	Scottsbluff - 2	July 29	Aug. 3	Severe
1958	Alliance	July 22	July 23	Severe
	Hay Springs	July 22	July 23	Severe
	Mitchell	July 22	July 23	Severe
	Scottsbluff - 1	July 22	July 23	Severe
1959	Hay Springs	None	None	None
	Mitchell	None	None	None
	Scottsbluff - 1	None	None	None
	Scottsbluff - 2	None	None	None

[a]Scottsbluff - 1 = elevation comparable with Scottsbluff Exp. Sta., 4040 feet.
[b]The disease was not present in some fields and traces were found in others.
[c]Scottsbluff - 2 = North Platte River lowlands interpreted from data taken at Barbour farm, 3860 feet or 180 feet lower than the Experiment Station.

During 1954 the hygrothermographs were started on July 27, a late date for recording the meterological data associated with the early development of the pathogen.

On August 17, on the basis of data recorded on the charts of the preceding 3 weeks, zero to slight blight development was predicted for Alliance, Hay Springs and Scottsbluff for the week ending August 23. Six days after the forecast was issued, a trace of late blight was reported from a potato field near the North Platte River at Scottsbluff-2. No blight was observed at the other two stations.

Heavy blight development in the river valley area around Scottsbluff-2 to zero in the Alliance-Hay Springs area was forecast September 10. Six days later this prediction was confirmed by a report of late blight in most of the potato fields in the North Platte Valley in the Scottsbluff-2 area, with epiphytotic development in some fields[4]. Blight was not reported from Alliance, Hay Springs, or Scottsbluff-1 station, nor was the disease reported from these three stations throughout the season.

During 1955 nine forecasts were issued for the four stations in western Nebraska. No blight was predicted for three stations (Alliance, Hay Springs and Scottsbluff-1). However, slight blight development was predicted for the Scottsbluff-2 weather-late blight station near the North Platte River (Barbour's farm) in eight of the forecasts. During seven of the following 8 weeks, slight blight was forecast and that amount developed in the field housing the instrument and in nearby fields. In other fields, 6 miles east of the instrument, moderate amounts of blight developed. However, the warnings were issued in ample time for control measures to be instituted and the slow, steady development of the disease was predicted far in advance of its discovery.

As predicted, the disease did not develop at Scottsbluff-1, Alliance or Hay Springs.

During 1956 five forecasts were issued for four stations in western Nebraska. The first, issued August 11, warned of a possible trace of blight in the Scottsbluff area but predicted no development at the Alliance, Hay Springs and Experiment Station locations. On August 24 active late blight was found in part of an unsprayed field 25 miles southeast of the Scottsbluff-2

[4]A written communication from George Stachwick, former Extension Horticulturist, University of Nebraska.

FIGURE 1. The weekly late blight severity values, the late blight intensity, and the date blight reported at five stations in Nebraska during the period 1952-1959.

weather station.

The accuracy of the predictions was indicated by the final results of the season. No late blight was found at any of the stations in western Nebraska. Presumably, the three successive weeks of no favorable periods at the beginning of the season inhibited the fungus at the Scottsbluff-2 station.

In 1957 data were obtained from five stations in western Nebraska. The first blight warning for western Nebraska was issued July 29 for the 7-day period ending August 3.

During the season four warnings were issued for each station. Late blight was found on August 3 at Alliance, the Scottsbluff Experiment Station, low fields at Scottsbluff, and Mitchell. All warnings were issued in time for the growers to initiate control measures. Some growers heeded the warnings, applied fungicide, and contained the fungus; others did not and sustained heavy losses. The latter was especially true in the Alliance and Scottsbluff areas.

In 1958 a blight warning was issued on July 22 and the disease was reported on July 23, a day after the warning was released. In this instance the hygrothermograph data indicated correctly the early incidence of disease but were not available for prompt analysis and forecast. However, the warning was issued in time to apply adequate control measures. Weekly forecasts of anticipated blight development were issued throughout the season. Late blight was severe in the western potato area at all locations.

In 1959 blight warnings were issued for western Nebraska from July 2 to July 30. After July 30 warnings were dropped for all areas except Hay Springs because of unfavorable weather conditions. No blight was reported during the season at Scottsbluff, Alliance or Mitchell. Subsequent weather conditions at Hay Springs became unfavorable for late blight and, accordingly, blight warnings were dropped. Late blight did not occur there.

DISCUSSION AND CONCLUSIONS

Temperature-relative humidity criteria effectively provided an accurate basis for forecasting the incidence and development of late blight in the irrigated potato fields of western Nebraska. In some instances the first blight warning was issued only a day ahead of the first report of blight incidence, whereas in other instances 28 days elapsed between the first warning and the first reported observation of the disease.

The discrepancy in time may be attributed to one or a combination of four situations de-

pending upon the time lapse between the forecast and the reported incidence: 1) the time lapse between emergence of the potato shoots and the initiation of hygrothermograph-data collecting in the field; 2) the time lapse between occurrence of favorable periods for late blight development and the receipt of the hygrothermograph charts for analysis; 3) the obscurity of the disease in the early stages; or 4) the suppression of the fungus by fungicides which retard the development of early obvious symptoms.

The relation of the "blight severity values" to the date late blight was first reported and the final late blight severity at the close of the season at five locations is illustrated in Figure 1. In general, the severe and moderate blight intensities were associated with a greater frequency and weekly total of "blight severity values." The localities reporting no or trace blight conditions seemed to have one condition in common, that is, there were periods of 2 to 3 weeks of unfavorable blight conditions during the season. During one of the trace blight years, the 3-week period occurred as two consecutive weeks and a single weekly period.

The data in Figure 1 illustrate the importance of setting up the recording instruments during the emergence period. For example, during 1954 at Scottsbluff-2 and in 1958 at Alliance, Mitchell and Scottsbluff-1, late blight was found during the week the hygrothermographs were set out. Hence, conditions during the critical period leading to the appearance of late blight were not recorded.

The situation at Hay Springs in 1959 was interesting in that the meteorological data indicated that late blight was in the area and it may not have been seen.

The failure of the fungus to survive three successive weeks of adverse conditions was illustrated by its failure to develop at the Scottsbluff-2 station in 1956 at the onset of favorable conditions subsequent to the adverse period. Thus far, the above-mentioned phenomenon seems to be a "rule of thumb."

These results illustrated the usefulness of interpretations of hygrothermograph data in predicting the early, unseen growth and development of the late blight fungus in the field. The early stage is the most insidious phase in the life history of the fungus because the pathogen "smolders" unseen in the foliage, thus disarming the grower of the necessity of applying control measures. An awareness of the "smolder" phase enables the grower to plan his attack instead of guessing the "right time" to spray.

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CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE AND NEBRASKA AGRICULTURAL EXPERIMENT STATION, LINCOLN

SEED TRANSMISSION OF SAFFLOWER VERTICILLIUM WILT FUNGUS[1]

M. L. Schuster and D. S. Nuland[2]

The appearance of Verticillium wilt of safflower in 1958 at a Scottsbluff Experiment Station nursery, not known to harbor the disease, indicated that seed transmission may have been instrumental in the introduction of the causal fungus. Diseased plants in the irrigated nursery, which is located on Tripp very fine sandy soil, were isolated from other plants throughout the plot. This further suggested seed transmission. Local dissemination of Verticillium type diseases can occur by means of diseased vines and soil transfer from wilt-infested fields. An attempt has been made to ascertain whether the Verticillium wilt fungus under certain conditions may be transferred in the seed. Results thus far obtained are included in this report.

Other related vascular diseases have been studied as to the importance of seed transmission. Freely exchanged seed lots are grown in the safflower nursery, which practice may provide a means of introducing Verticillium-infected seed. Seed infection by Verticillium has been studied in several crops. Researchers are in disagreement as to the importance of seed transmission of Verticillium in cotton, tomato and eggplant. Apparently local conditions affect the incidence of seed infection by Verticillium. This subject was reviewed recently by Burton and de Zeeuw (1).

The only previous report, to the authors' knowledge, of Verticillium wilt of safflower is from Russia by Golovin (2)[3]. He noted that the lower leaves of infected plants turn yellow, followed by a general yellowing of the entire plant. We noted that a bright yellow coloration of Verticillium-infected plants is typical of the disease in the field. This coloration was commonly expressed in leaves on one side of the midrib, while the other side remained normal. The change in color from green to yellow began in both the older and younger leaves. A unilateral yellowing of the plant may occur and later the entire plant may be involved. The typical flaccid type wilt is not common in safflower at flowering time.

Golovin (2) stated that brown discoloration of the vascular system was evident only in the roots of Verticillium-infected safflower. Our observations in the field and of Verticillium-inoculated plants in the greenhouse definitely show vascular discoloration in the aerial portions as well as in the roots. At times this was expressed to the degree that when side branches were removed by hand, the brown or dark brown vascular areas became visible macroscopically. In fact, the very pronounced discoloration of the vascular tissues of the aerial portions of the plant in the nursery also suggested the possibility of seed infection by the pathogen.

The disease may make its appearance very early in the development of the plant, but is quite noticeable during the flowering period. Infected plants are frequently stunted and the yield is reduced as evidenced by poorly filled seed. Seed harvested from infected plants were either normal (plump and white to cream in color), shriveled and purple, or plump with purplish areas. Seed symptoms also suggest possible seed infection by the fungus.

After consistent isolation of Verticillium from stems and branches of plants with the previously described symptoms, seven Verticillium-wilted plants were tagged during their flowering period in 1958. Upon maturity seeds were harvested from these as well as from healthy plants. Heads of each plant were harvested individually.

Preparatory to plating on 2% potato-dextrose agar, the seeds were treated in 1% sodium hypochlorite for 15 minutes or in 2% concentration for 5 minutes, and then rinsed in sterilized distilled water. The percentage germination of seed on agar or the percentage expression of Verticillium was not affected by the time of treatment or concentration of sodium hypochlorite. Five seeds from each safflower head were treated and plated on the agar in Petri dishes. The number of seeds plated from seven respective plants labeled consecutively 1 to 7 was 80, 25, 45, 55, 70, 50 and 45, making a total of 370 seeds. Twenty-two seeds from healthy plants were used as controls.

From the seed isolation test we found that 9% of the seed from wilted plants contained Verticillium. Two other fungi were commonly obtained from the seed: Alternaria species (28%) and a non-sporulating dark mycelial type (38%). These two fungi were also isolated from seed of "healthy" plants. Verticillium was not isolated from "healthy" seed. From previous saf-

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[3]The authors acknowledge translation of this note by Dr. A. Mazurak, University of Nebraska.

flower rust studies, Alternaria was found to be a common seed contaminant.

Seed from safflower grown in greenhouse soil infested with each of four Verticillium isolates 1-1, 2-7, 3-3 and 315 yielded 44, 15, 13 and 21% of the fungus. Plants selected for these isolations exhibited Verticillium wilt symptoms. The average of 23% of Verticillium isolations was from a total of 177 seed. Verticillium was not obtained from 65 seeds of uninoculated controls.

Pathogenicity tests were conducted in the greenhouse with four cultures of Verticillium representing three monosporic lines isolated from seeds of three infected plants and one line from the stem of an infected plant. Inoculum grown on agar was incorporated in the soil. Roots were wounded 1 week after inoculation. The four isolates were pathogenic on the N-8 variety of safflower but differed in the percentage of infected plants and intensity of vascular discoloration. Cultures 1-1, 2-7 and 3-3 caused wilt symptoms in 23, 85 and 100% of the plants inoculated. The stem isolate 58-3 induced wilt symptoms in 29% of the plants. Verticillium was isolated from infected plants without difficulty.

A pathogenicity test using three monosporic lines of Verticillium from safflower seed and a potato isolate was conducted on six safflower varieties. The results show variability in varietal reaction to the fungus and in virulence of the pathogen (Table 1). Cross inoculation with safflower isolates proved pathogenic for potatoes. Cultures 1-1, 2-7 and 3-3 caused 83, 83 and 57% wilt in the potatoes inoculated by a modified toothpick method (3). The bright yellow coloration of infected safflower was not so pronounced in the greenhouse tests as under field conditions. Infected plants usually appeared dull yellow or grayish yellow in color.

Table 1. Percentage of wilted plants of six safflower varieties upon inoculation with different Verticillium isolates.

Culture No.[a]	Safflower variety					
	N-3	N-6	N-8	N-10	P-1	N-4036
1-1	6	0	0	16	12	0
2-7	5	0	20	44	8	0
3-3	95	100	75	85	50	55
315	22	54	33	53	16	54
Check	0	0	0	0	0	0

[a] Culture 315 from potato; others from safflower seed.

DISCUSSION AND SUMMARY

The results are interpreted as definite evidence that Verticillium is carried in safflower seed.

The conditions of the experiments may greatly exaggerate field conditions in that only seeds from wilted plants were used. But of a population of 370 seeds, selected for their greater probability of carrying the fungus, 9% showed presence of the pathogen. In greenhouse tests a larger percentage of seeds showed presence of the Verticillium fungus. If seed infection could result from aerial contamination, the seed from uninoculated controls should contain Verticillium since they were grown in proximity to the inoculated plants. The infected seeds introduced into the soil would serve as a potential source of primary inoculum. Since this fungus can survive in the soil, new areas of infestation may be established by Verticillium strains pathogenic for safflower. Although shriveled seed from wilted plants may be eliminated to a great extent during seed processing, infected seed of normal plumpness may act as a source of inoculum into wilt-free soil suitable for the establishment of the fungus.

The fact that Verticillium from potatoes is pathogenic for safflower and isolates from safflower infect potatoes emphasizes the potential importance of this disease in irrigated areas of western Nebraska. Three safflower seed isolates caused wilt in a large percentage of the potato plants inoculated. Other crops may also prove susceptible to strains already existent in that area.

The Verticillium isolates from both the safflower and potato are of the pseudosclerotial type. Some workers assign the isolates producing pseudosclerotia to the species Verticillium dahliae Kleb. Golovin (2) identified the isolate from safflower to this species. Researchers have demonstrated the morphological instability of monosporic cultures. Because of this fact the writers are not inclined to assign the Verticillium isolates to either V. dahliae or V. albo-atrum Reinke & Berth.

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NEBRASKA AGRICULTURAL EXPERIMENT STATION, LINCOLN

THE ASSOCIATION OF A RED-LEAF CONDITION OF PEAR TREES WITH PEAR DECLINE[1]

Earle C. Blodgett and Murit D. Aichele[2]

Abstract

Red fall coloration of pear tree foliage can be caused by factors such as: 1) inherent incompatibility of scion and stock, 2) root injury, 3) girdling. In pear trees showing early red fall color and growing in experimental plots at Prosser, Washington these conditions were not present. Examination of roots under these trees showed varying degrees of root degeneration. The association of this root damage with the early red fall color and the subsequent lack of growth of trees the following spring gives evidence that red-leaf in pear trees is associated with the decline factor.

Several diseases are characterized by the development of premature red coloration of foliage. One example is yellow-red virosis (3) of peach, which is also associated with red-leaf of chokecherry (X disease complex). Another is the red coloration in sweet cherry (in particular Bing and Van varieties) affected with the K & S virus (4). More common is the red-leaf development in girdled pear trees or in individual branches where fire blight cankers are present. A red-leaf condition may also exist when certain pear varieties are budded or grafted on incompatible rootstocks, as with Bartlett scions on quince.

Probably the first mention of an unusual red-leaf condition in declining pear trees was made by Degman in 1954 (2). By 1954, many if not most of the pear orchards in central Washington were showing various degress of red leaf coloration during early fall. As early as 1951 the senior writer was impressed by, and took Kodachromes of, the brilliant coloration of Yakima pear orchards but did not then connect this phenomenon with pear decline.

The first clue of the possible significance of red leaf coloration in pear foliage was its development in several 2-year-old nursery trees in experimental pear nursery plots at Prosser, Washington in the fall of 1958. While checking commercial yearling pear nursery stock in nursery blocks in October 1958, the writers observed that a rather high percentage of the nursery trees showed varying degrees of dull to bright red leaf coloration. This red-leaf occurrence did not fit soil or moisture variations nor the pattern of budstick use in the nursery rows. Both Bartlett and Anjou varieties budded on domestic Bartlett and imported French pear seedling rootstocks were involved. In the experimental pear nursery plots at Prosser, more red-leaved trees occurred when the scions were budded or grafted on seedlings of Pyrus ussuriensis and/or P. serotina.

Forty-two of the trees from the experimental nursery plots and 40 from one of the commercial nurseries were marked with paint and the severity of the symptoms recorded. Trees with the reddest foliage tended to be smaller. These trees were planted in experimental plots in March 1959. Some, but not all, of these trees turned red during the fall of 1959.

Blodgett and Aichele (1) have described some phases of the work on pear decline with special attention to the planting of different scion/rootstock combinations at Prosser in 1958 and 1959. Some of the trees were reported to have wilted and died in the late summer of 1959. Wilting was confined to pear varieties on P. ussuriensis and P. serotina seedling rootstocks.

Shortly after the first of September 1959, several of the trees in these plantings were turning red. Most, but not all, of the trees showing symptoms were on P. ussuriensis and P. serotina rootstocks. The entire planting, consisting of several experimental plots, was mapped four times and changes in color noted. The data for Roza experiments 1A and 1C are shown in Figure 1.

On October 6, 1959 a portion of the fibrous root system of each of 70 trees in these experimental plots was taken and critically examined. The observations made on 33 of these trees are presented in Table 1. Trees with red leaf coloration showed much more degeneration of the fibrous root system than did those free of red-leaf symptoms. Small roots under healthy trees, including those of some trees on P. ussuriensis, were normal in appearance.

[1]Scientific Paper No. 2032, Washington Agricultural Experiment Stations. Work was conducted under Project No. 1337.

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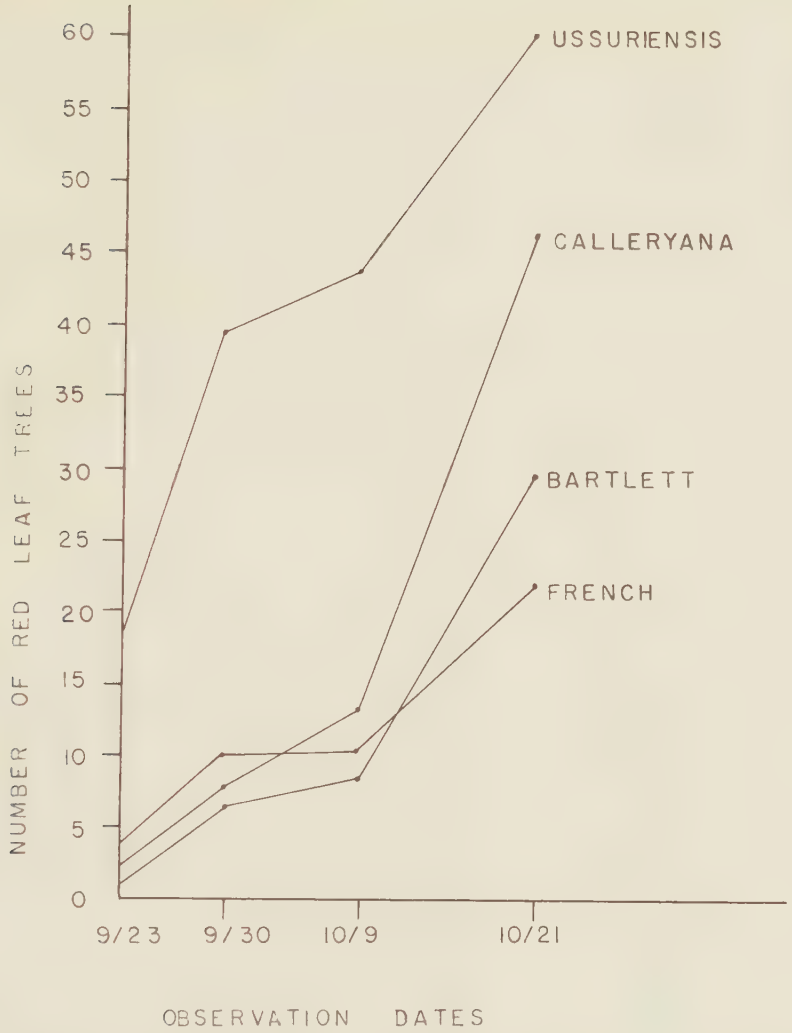

FIGURE 1. Number of pear trees on four rootstocks showing a red-leaf condition during late summer 1959 in Roza plots 1A and 1C, Prosser, Washington. Total trees each on Bartlett, Ussuriensis, and Calleryana = 96. Total trees on French = 84.

One of the most interesting observations was the close correlation of root condition and leaf coloration with the subsequent growth of trees the next season. Trees with early and severe red fall color in conjunction with poor roots made little or no growth the following spring.

OTHER OBSERVATIONS

On September 22, 1959 while visiting a nursery west of Portland, Oregon in company with Mr. Richard Hemmerling of the Oregon State Department of Agriculture, the senior writer commented on the brilliant red-leaved condition of several 2-year-old pear trees. We were advised that the trees were about to die because of woolly aphis root injury. We dug one of the trees, and although no aphis were found there was evidence that they had been present. The roots, including those of medium size, were dead. Other red-leaved nursery trees were examined on October 29 in the same nursery and live root aphis were found. Perhaps as many were also found on normal appearing trees in the same rows. At tree digging time during November 1959, the junior writer and Dr. Marvin Brunson, Entomology Section, Agricultural

Table 1. The comparative root condition and tree ratings associated with a red coloration of foliage on 33 two-year-old Bartlett pear trees growing on four different rootstocks in plots at the Irrigation Experiment Station, Prosser, Washington, October 1959.

Tree location block-row-tree	Rootstock	Leaf color	Root condition[a]	Tree rating June 1, 1960[b]
1 - 3 - 12	Ussuriensis	normal	1	1
1 - 2 - 5	Ussuriensis	normal	1	Q.D., 7/19/60
1 - 16 - 5	Ussuriensis	normal	1 - 2	Q.D., 7/5/60
1 - 14 - 8	Ussuriensis	slight red leaf	2 - 3	2
1 - 15 - 5	Ussuriensis	slight red leaf	3 - 4	2
1 - 4 - 5	Ussuriensis	slight red leaf	2 - 3	3
1 - 3 - 11	Ussuriensis	slight red leaf	2 - 3	3
1 - 2 - 11	Ussuriensis	slight red leaf	2	3
1 - 15 - 8	Ussuriensis	red leaf	3	3
1 - 15 - 3	Ussuriensis	red leaf	4	4
1 - 5 - 12	Ussuriensis	red leaf	3	4
1 - 23 - 7	Ussuriensis	red leaf	3	4
1 - 17 - 1	Ussuriensis	quick decline	4	dead
1 - 14 - 5	Ussuriensis	quick decline	4	dead
1 - 6 - 9	Calleryana	normal	1	1
1 - 1 - 9	Calleryana	slight red leaf	1	1
1 - 6 - 12	Calleryana	slight red leaf	2	1
1 - 12 - 4	Calleryana	slight red leaf	2 - 3	3
1 - 16 - 6	Calleryana	slight red leaf	2 - 3	1
1 - 7 - 14	Calleryana	slight red leaf	3	1
1 - 9 - 3	Bartlett	normal	1	1
1 - 2 - 2	Bartlett	normal	1	1
1 - 9 - 6	Bartlett	slight red leaf	1	2
1 - 22 - 14	Bartlett	slight red leaf	1 - 2	3
1 - 13 - 9	Bartlett	slight red leaf	2	3
1 - 13 - 2	French	normal	1	1
1 - 2 - 8	French	normal	1	1
1 - 2 - 7	French	slight red leaf	1	1
1 - 19 - 12	French	slight red leaf	1 - 2	3
1 - 23 - 8	French	slight red leaf	2	2
1 - 1 - 7	French	red leaf	1 - 2	4
1 - 21 - 7	French	red leaf	2	3
1 - 15 - 2	French	red leaf	2 - 3	3

[a] A root sample from each tree, consisting of a portion of root approximately 1/4 inch in diameter and several small rootlets, was observed under a low-power dissecting microscope. A number 1 rating represents a healthy root which had been growing rapidly; the outer bark is splitting and sloughing off; the phloem, cambium, xylem and rootlets are normal. A number 4 rating represents a root showing no splitting of the outer bark and with only a few shriveled, brown to black rootlets and with black phloem and cambium and brown xylem. Numbers 2 and 3 represent intermediate stages. Several other less obvious differences entered into the ratings.

[b] Throughout the pear decline work, tree ratings have been designated by numbers 1 to 5. 1 = normal foliage, very vigorous growth; 2 = normal foliage, good growth; 3 = smaller, lighter colored leaves, poor growth; 4 = very poor foliage, very poor growth; 5 = tree nearly dead, no growth; Q.D. = quick decline. All 33 trees rated number 1 in growth during the 1959 growing season.

Research Service, United States Department of Agriculture, stationed at Yakima, Washington could find no infestations of woolly aphis on pear trees in two Washington nurseries where red-leaf was common.

On October 28, 1959 the writers examined Anjou and Bartlett pear orchards in the vicinity of White Salmon, Washington. In some orchards most of the leaves had fallen; in others there was the same general pattern of strikingly red-leaved trees with a few to many normal green-leaved trees nearby. Adjacent plantings varied widely in the amount of red color present.

Orchards were observed in the Vancouver, Washington area and counts were taken of red-leaved trees in young and older orchards. The data are shown in Table 2.

Table 2. Red-leaved trees in five pear orchards in Vancouver, Washington area.

Orchard No.	Age of trees (in years)	Trees with red leaf	Total trees	% with red leaf
1	1	38	294	13
2	5 - 6	153	370	41
3	3	25	126	20
4	4	30	70	43
5	12	16	304	5

In orchard No. 2 trunk and branch cankers, cause undetermined, were present and may account for some of the red leaf coloration.

The senior writer observed pear orchards in Oregon and California between November 2 and 11 in 1959. Varying amounts of red-leaf were encountered in many orchards in all districts visited. One point may be significant: red-leaf in nursery trees was rare in California and less frequent in Oregon than in Washington.

Most of the commercial pear nursery stock in the three States is propagated on domestic Bartlett seedling rootstocks which are grown in Washington and Oregon.

DISCUSSION

For the past 2 years red coloration of pear leaves has received special attention in Washington. It seems unlikely that leaves on normal, healthy Bartlett pear trees turn red at any time. In the experimental plots at Prosser red-coloring is more common when trees are propagated on oriental rootstocks, namely P. ussuriensis and P. serotina, than when propagated on domestic Bartlett or imported French pear rootstocks. Since there are no fire blight cankers or other evidence of girdling, the red-leaved condition of trees in these plots cannot be attributed to such factors. Red coloration is good evidence of girdling, root degeneration, or a combination of these two factors. As shown in Table 1, red-leaf was associated with a degeneration of the root system and trees exhibiting these symptoms showed slow decline the next growing season. It seems reasonable to conclude that red coloration of pear leaves is closely associated with the type of pear decline occurring in the plots at Prosser.

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SEPTIC CULTURE OF CERATOCYSTIS ULMI ON ELM WOOD

Richard Campana and Martin Rosinski[1]

The accepted practice for positive diagnosis of Dutch elm disease is the isolation in pure culture of the causal organism (Ceratocystis ulmi (Buis.) C. Moreau), and the identification of its conidia produced in sterile agar in Petri plates.

In the course of a study of host-parasite relationships in Maine, a simplified method for culturing the Dutch elm disease fungus on elm wood was tested during the summer of 1960. The method is a modification of the moist-chamber technique described by Epstein (2), and depends on production of coremia as indicated by Hart (5). However, it differs significantly in its simplicity, requiring no sterile technique nor special prepared media, thus being especially suitable for field culture.

For several years the writers and many others have observed that Petri plates of potato-dextrose agar (PDA) contaminated with bacteria or fast growing molds failed to yield the Dutch elm disease fungus unless coremia developed on wood chips. In some cases coremia have been discovered inadvertently on chips of infected elm wood either surrounded by bacteria or overgrown with molds at the time plates were to be discarded as negative. When streaked with a needle on PDA such coremia have invariably produced pure cultures of the Dutch elm disease fungus. Fenner and Liming (4) reported in 1947 that several saprophytic fungi and aerial contaminants may overrun and mask coremia of C. ulmi on elm wood. Dothiorella ulmi and a species of Cytospora were mentioned specifically in limiting development of coremia of C. ulmi, but they were not reported to prevent coremial development completely.

In contrast, in studies[2] by the senior author on the ability of C. ulmi to produce coremia in mixed cultures with other vascular pathogens of elm, with an elm inhabiting bacterium, and with common aerial contaminants, he found that C. ulmi produced coremia on sterilized elm wood in the presence of Dothiorella ulmi (Cephalosporium stage), Verticillium albo-atrum, Rhizopus nigricans, Aspergillus niger, one species each of Penicillium and Fusarium, and the bacterium. Although such cultures were frequently overrun or dominanted by the other organisms (except with the bacterium), C. ulmi produced coremia in every mixture with these organisms.

The production of well defined and easily recognizable coremia amid abundant and varied contaminants led the present writers to suspect that C. ulmi might be cultured and identified on wood routinely without aid of sterile methods.

METHODS

Inoculated Elm Stems: With no precautions for sterility, more than 900 inch-long elm sections of stems 1/4 to 1/2 inch in diameter from 143 inoculated trees were stripped of bark and placed either in vials sealed with corks or in Petri plates moistened with damp paper toweling. Since most of these elm sections were being tested for initial movement of the fungus following inoculation, they were not discolored. Plates and vials were kept at room temperature and examined daily over a period of 1 to 2 weeks for appearance of coremia.

Paired Comparisons: In a second series of observations, 456 paired samples from 228 trees from scattered locations throughout the State were tested to compare the conventional aseptic agar-plate method against a new septic wood-vial technique as described[3] (1). As before, with no precautions for sterility, bark was stripped from branch samples about 1/2 inch in diameter; discs about 1/2 inch long were cut with pruning shears, immersed about 1 minute in ordinary tap water, and then placed in glass vials which were immediately stoppered with tightly-fitting corks. Although most work was done in the laboratory, some vials were prepared in this way in the field.

Exposure to Water and Clorox: In another series of tests, infected elm discs were tested in vials: 1) with no water immersion; 2) following immersion from 15 to 60 seconds; 3) with a shallow film of water around the bases of discs. In addition, infected discs were tested after

[1]The writers acknowledge the aid of Mrs. Laszlo Darko, Mrs. Rupert Stafford, Mr. Donald Sylvester and Mr. Charles Micciche who, as technicians and students, assisted in the project.

[2]Unpublished observations by R. Campana as associate plant pathologist, Illinois Natural History Survey, 1956-58.

[3]This method was first described by the senior author in a talk before publishers of the Shade Tree Digest at the National Shade Tree Conference in Boston, Massachusetts in August 1960.

exposure to: 1) sterile water; 2) tap water; 3) dilutions of grocer's Clorox (sodium hypochlorite) in tap water ranging from 10 to 50%.

Exposure to Contaminants: To test the ability of C. ulmi to compete in presence of other vascular fungi of elm and common fast growing contaminants, various series of wood-vial cultures were arranged mixing C. ulmi with various fungi or bacteria. About 400 elm discs were prepared in vials as previously described except that half were tested with bark and half without. Half of the discs were from wood known to be infected with C. ulmi, and the other half were from noninfected, healthy wood.

A single drop of fungus spore or bacterial suspension of more than 1,000,000 per ml for each organism listed in Table 1 was placed on the upper flat surface of each naturally-infected disc. Each control disc was inoculated with a single drop of tap water. Half of the infected discs were then rinsed with tap water by filling, shaking and draining the vials. Each treatment with infected discs, including those debarked, with bark, rinsed, and non-rinsed, was replicated five times.

Table 1. Percentages of elm discs producing coremia of C. ulmi by dilution of inocula, type of wood, inoculation, and presence of bark.

	Inoculation minus rinse				Inoculation plus rinse				
	Infected wood		Healthy wood		Infected wood		Healthy wood		
Inoculum[a]	WB	WOB	WB	WOB	WB	WOB	WB	WOB	Mean
Control[b]	100	100	100	100	90	90	90	80	94
Verticillium	100	100	60	80	60	80	--	--	80
Rhizopus	100	100	100	100	100	100	100	100	100
Bacterium	100	100	100	100	100	100	100	100	100
Fusarium	60	100	100	100	80	100	100	100	92
Aspergillus	90	100	90	100	90	100	90	80	92
Alternaria	90	80	100	100	90	100	100	90	94
Dothiorella	60	80	80	100	90	100	100	100	89
Penicillium	0	10	100	100	50	100	60	100	65
Trichoderma	0	0	20	100	10	90	50	100	46
Mean	70	77	85	98	76	96	88	94	

[a] C. ulmi X each inoculum listed except for control.
[b] For C. ulmi-infected discs, water only; for healthy discs, C. ulmi only.

The remaining 200 healthy discs were inoculated with a single drop of mixed suspensions of C. ulmi and each of the organisms listed. Control discs were inoculated with C. ulmi only. Inoculated healthy discs were treated in a manner similar to infected ones, and all treatments were replicated five times. Discs were examined carefully under a stereo binocular dissecting microscope daily for presence of coremia for 3 weeks, and the percentages of discs yielding coremia of C. ulmi were recorded (Table 1).

RESULTS

Inoculated Elm Stems: Of 940 inoculated sections tested for movement of C. ulmi in elm stems, 447 (47%) produced coremia, mostly in 3 to 6 days, but in some cases in 7 to 14 days. Although most sections were not discolored, abundant coremia were produced. Sections with surface discoloration generally produced coremia in 3 to 4 days, whereas those with internal discoloration rarely produced them before 6 to 7 days.

Paired Comparisons: Of the 228 paired comparisons between aseptic-agar-plate and septic-wood-vial methods, 48 (23%) determinations of C. ulmi were made by the wood-vial method that were missed by the standard agar-plate technique. In some cases positive results with wood culture were not obtained for 3 weeks. However, about 80% of discs yielding coremia produced them in 3 to 6 days. Positive confirmation of C. ulmi was obtained by streaking coremial heads with an aseptic needle on PDA and obtaining pure cultures. In no case was C. ulmi isolated by the plate method where it was missed by wood-vial culture. Although in most of these cases plates had become contaminated with bacteria or fast-growing fungi, in some cases nothing grew from the chips. Species of both Dothiorella and Verticillium were each isolated by the plate method from single but different elm samples, the infected discs of which produced coremia of C. ulmi with the wood-vial method. This situation occurred on

four separate occasions from four separate samples. Since both Dothiorella and C. ulmi, and Verticillium and C. ulmi were previously isolated together on plates of PDA by the senior author, the wood-vial method may have an advantage in preventing the masking of C. ulmi by these and other vascular parasites where one of them occupies the same wood with C. ulmi.

Exposure to Water and Clorox: Where infected discs were tested against different moisture conditions, coremia of C. ulmi were produced with different degrees of success. Although coremia occurred to some degree under all conditions, they were only produced reliably or within 3 to 5 days when discs were immersed momentarily and drained. Without wetting, discs dried too quickly and coremia were slow in developing, developed sparsely, or did not develop at all. With free water, excessive bacterial contamination or fermentation either prohibited or slowed coremial development. Neither sterile water, tap water, nor weak dilutions of Clorox appeared to affect production of coremia. With stronger dilutions of Clorox, from 30 to 50%, coremial production was inhibited progressively.

In most tests coremia appeared regularly within 3 to 5 days on infected wood, but they appeared as soon or sooner on healthy elm discs inoculated with spore suspensions of C. ulmi. Although they appeared most abundantly on the upper cross-sectional surface of the disc, they appeared on all surfaces including the bottom one, and at times they were more abundant near the base or could only be detected on the bottom. In some cases they appeared in the watery extract around the disc at the bottom of the vial or even on the sides of the vial. However, data include only actual appearance of coremia on wood.

Exposure to Contaminants: The data in Table 1 indicate that C. ulmi is able to compete favorably against the bacteria and fungi tested on and/or in elm wood. Several fungi (Fusarium, Dothiorella, Penicillium and especially Trichoderma) inhibited production of coremia of C. ulmi from naturally-infected wood, but only Penicillium and Trichoderma had much influence on spore suspensions of C. ulmi on healthy elm discs. The presence of bark on discs definitely inhibited development of coremia, especially in association with Penicillium and Trichoderma. These fungi, with Rhizopus and Aspergillus, appeared to be common, aggressive inhabitants of elm bark. Where naturally infected discs were rinsed with tap water following exposure to contaminants, or where discs inoculated with mixed suspensions were rinsed, development of coremia increased significantly in the presence of those fungi (Penicillium and Trichoderma) which inhibited coremia most strongly.

DISCUSSION

Because of the ease with which C. ulmi can be cultured and identified on infected elm wood without prepared media and without sterilization, the wood-vial method may be used effectively either in the laboratory or the field, but only with caution. Although elm wood with bark removed appears to be relatively free of bacterial and fungus contaminants, data is unavailable on possible antagonism to C. ulmi by fungus contaminants not yet tested, some of which may colonize elm bark and wood, or which may be aerial contaminants. Other Graphium species are reported to colonize elm (4, 6, 7). Fenner and Liming (4) reported at least six such species; and Shafer and Liming (6) reported that coremia of other fungi resemble those of C. ulmi in at least some aspects. It is also possible that certain strains of the Dutch elm disease fungus may not always produce coremia under the conditions described, although the writers were unable to find evidence to suggest this in the literature. Shafer and Liming (6) discussed production of coremia by 53 strains of C. ulmi without such an indication.

In addition, it is recognized that a non-sterile, moist chamber is an invitation to an unknown variety of microbial life, some of which may influence survival of C. ulmi in a manner extremely difficult to ascertain. We have already noted evidence of microbial succession on elm disc cultures in this study, and it is most probable that such cultures harbor unknown or undetected fungus-digesting bacteria.

Since it was anticipated that ordinary contamination through septic handling would approximate the rinsing treatment tested here, the data suggest that neither Trichoderma, Penicillium nor other fungi may pose as great a threat to production of coremia as indicated without rinsing, especially when bark is discarded from discs.

Observations reported here suggest that relatively clean septic cultures can be made with vials, that a mild Clorox rinse may possibly be equally as successful as a tap water rinse, and that this method offers an easier and possibly more accurate method for detecting the Dutch elm disease fungus than the plate-culture method. This method is not often as fast as the plate culture, however, and it cannot be guaranteed to produce other vascular pathogens of elm for positive identification. To date it has been possible to identify the Verticillium fungus on wood but not Dothiorella.

Until more information is available it is suggested that elm samples from which wood-vial cultures are made be held in refrigeration for plate-culture test, pending dubious or negative results. With this approach many hundreds or thousands of plate cultures may be eliminated. In addition, many hundreds of cultures could be made easily by commercial arborists and municipal foresters for confirmation by mycologists and pathologists, thus relieving State university facilities and staff from the cumbersome, time-consuming routine of mass isolations by plate culture. With further evidence on microbial relationships in mixed cultures it may be possible to extend to those most directly concerned with individual diseased trees more reliable information on the use of the wood culture.

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MAINE AGRICULTURAL EXPERIMENT STATION, ORONO

SPORULATION OF PYRENOPHORA BROMI ON ARTIFICIAL MEDIA[1]

S. A. Tamimi and G. M. Dunn[2]

Abstract

Conidial production was very poor among 257 leaf-lesion isolates of Pyrenophora bromi. Various media and light treatments did not significantly increase conidial production, although slight increases were obtained on "V-8" agar. Approximately half of the isolates grown on a grain medium produced mature ascospores. None of approximately 30 single ascospore isolates sporulated on either PDA or grain.

One of the destructive foliar diseases of bromegrass (Bromus inermis) is brown leaf-spot caused by Pyrenophora bromi Drechs. Natural infection varies widely in different years and locations. Artificial inoculation may therefore be of considerable value in selection for resistance.

Various workers (3, 4) have encountered difficulties in the production of conidia and ascospores on artificial media. An excessive period of time, approximately 4 to 5 months, is also required for production of ascospores. The studies reported here were made to improve sporulation of this organism.

LITERATURE REVIEW

Chamberlain and Allison (3) produced ascospores on potato-dextrose agar (PDA), after incubation for 4 weeks at room temperature, then for 3 months at 4° to 12° C. They reported that conidial formation was very sparse on agar. Carter and Dickson (2) produced conidia on soybean agar. Carnahan and Graham (1) produced ascospores on a wheat-oat mixture, after incubation at 20° for 4 weeks, then at 5° for 16 weeks. Consistency of sporulation was not discussed in previous work.

MATERIALS AND METHODS

Leaf-lesion and single ascospore isolates were utilized in this work. The former were obtained by plating sections of infected bromegrass leaves on PDA. Mecuric chloride (0.001% for 1 minute) was used as a sterilizing agent. Typical mycelium of P. bromi was reisolated, and growth from one leaf-lesion was considered an isolate.

Single ascospore isolates were obtained from perithecia which had overwintered in the field. Perithecia were collected from the leaves in February and March. Two to three perithecia were placed on a sterilized slide and crushed gently with a sterilized needle. A few drops of acidified, sterilized, distilled water were added to the crushed perithecia and stirred thoroughly. Pieces of perithecial tissue were removed from the spore suspension. Slides were checked under the microscope to determine the presence of ascospores in the suspension.

Thin films of filtered agar were poured in Petri dishes and allowed to solidify. Triangles (5 mm in size) were drawn on the outside of the dishes. Droplets of the spore suspension were transferred to the agar plates with a flamed glass rod, and the triangles were used for locating the droplets on the agar. The plates were incubated at room conditions (20° to 28° C) for about 4 hours, then checked under the microscope (8X). The agar around a germinating spore was cut with a sterilized needle, re-examined to insure the presence of only one spore on the agar block, then transferred to a Petri dish containing PDA.

Ascospore Production: Isolates of P. bromi were grown on a wheat-oats mixture as described by Carnahan and Graham (1) or on PDA for ascospore production. In the following

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tests the grain cultures were incubated for about 4 weeks and agar cultures for 2 weeks at room conditions. They were then exposed to cold treatments as described below:
Test 1: Ten leaf-lesion isolates were grown in 10 flasks containing a (2:1) wheat-oats medium. Also, mycelia from a mixture of 2 to 4 isolates were grown in six other flasks. Eight of the 10 isolates were also grown on PDA. The 16 flasks and 8 dishes were exposed to 6° C for 30 days, then to -12° for 33 days, then to 6° for 20 days. The cultures were then checked three times for spores at weekly intervals. The objective was to reduce the incubation period by exposure to freezing temperature.
Test 2: The 10 isolates used in test 1 were grown on PDA and exposed to 6° C for 10 weeks. The dishes were then checked twice for spores at weekly intervals.
Test 3: Twenty-eight leaf-lesion and 2 single ascospore isolates were grown on grain. Thirty flasks were inoculated with a single isolate each, and 30 flasks were inoculated with a mixture of 2 to 4 isolates. All flasks were exposed to 6° for 14 weeks, and were then checked four times for ascospores at weekly intervals.
Test 4: Eleven single ascospore isolates were grown on PDA separately and in various combinations. They were exposed at 4° C for 12 weeks.

Conidial Production: A total of 257 leaf-lesion isolates were obtained from infected leaves collected from several fields during the spring, summer and fall of 1958. They were grown on PDA for about 2 weeks, then were checked for conidia. Twenty-two single ascospore isolates were also grown on PDA.

The following additional tests were conducted:
Test 5: Seven leaf-lesion isolates which produced a few conidia on PDA were grown on 1) PDA, 2) "V-8" (vegetable juice) agar, 3) malt extract agar, 4) lima bean agar, and 5) bromegrass juice agar. In this test, 105 Petri dishes were inoculated with approximately equal amounts of mycelium, then were divided into three lots, and treated as follows: (a) placed under fluorescent light for 6 days, then at room conditions (20° to 28° C, and daylight) for 9 days; (b) incubated at room conditions for 15 days; and (c) incubated at 12° for 10 days, then at room conditions for 5 days.
Test 6: Four leaf-lesion and one single ascospore isolate, none of which produced conidia on PDA, were grown on "V-8" agar, bromegrass juice agar, and PDA. The dishes were divided into two lots, and treated as follows: (a) grown at room conditions for 2 weeks and (b) exposed to continuous fluorescent light for 1 week, then to room conditions for 1 week.

Several other tests were conducted involving a few isolates, with emphasis on light treatments and periods of incubation. The light treatments were: continuous fluorescent light, sunlight, ultraviolet light and darkness. Cultures were checked for conidia after 1, 2, 3 and 4 weeks of incubation.

RESULTS

Leaf-lesion isolates were obtained in 8 to 12 days after plating on agar. Remarkable differences were observed among these isolates in rate of growth, colony type, and size and number of sclerotia or perithecial initials (Fig. 1). Cultures of P. bromi were obtained from approximately one-third of the leaf-sections.

Single ascospore isolates differed markedly in color of mycelia. Most isolates produced no sclerotia on agar or grain (Fig. 2), while others varied widely in type and amount of sclerotia.

No ascospores were observed in the flasks or Petri dishes of test 1. In test 2, one isolate was discarded because of contamination. Four of the remaining 9 isolates produced mature ascospores. These isolates were only checked twice because of drying of the agar.

Eighteen flasks were discarded in test 3 because of contamination or lack of perithecia. Ten of 21 leaf-lesion isolates incubated singly produced mature ascospores, and immature asci were observed in four other flasks. Also, 10 of 20 flasks inoculated with a mixture of 2 to 4 leaf-lesion isolates produced mature ascospores, and asci were found in three other flasks. The remaining flasks did not produce asci or ascospores. The single ascospore isolates were discarded because no perithecia were observed in them.

No ascospores were observed in any dishes of test 4, and the cultures were discarded after checking once for spores because of contamination or drying of the agar.

The majority of the 257 leaf-lesion isolates did not produce conidia on PDA when incubated under room conditions. A limited number of them produced conidia sparsely, but none produced abundant conidia. None of the single ascospore isolates produced conidia.

FIGURE 1. Three leaf-lesion isolates of P. bromi grown on PDA and grain. Note variation in rate of growth on agar, and in amount of perithecia on grain.

FIGURE 2. One single ascospore isolate (left) and a leaf-lesion isolate (right) grown on PDA and grain. Only the leaf-lesion isolate produced sclerotia.

Leaf-lesion isolates which produced a few conidia on PDA were grown on different media and exposed to various environmental conditions in test 5 to increase conidial production. In general, the increase in sporulation was not significant. Best results were obtained on "V-8" agar incubated at 12° C for 10 days, then at room conditions for 5 days.

Isolates which produced no conidia on PDA did not sporulate under the various conditions imposed in test 6.

Several other tests involving variation in media, type and duration of light, and periods of incubation were ineffective in increasing production of conidia.

DISCUSSION

Large differences were observed among leaf-lesion isolates of P. bromi. Approximately one-half of the isolates grown on a grain medium produced mature ascospores. In a previous test at New Hampshire, 16 flasks of single, leaf-lesion isolates did not sporulate, while one grain flask inoculated with several isolates produced mature ascospores. This suggested that mixtures of isolates might improve sporulation of this fungus. However, mixtures of isolates in test 3 were not effective in increasing sporulation.

Conidial production was generally very poor, although slight increases were obtained under certain conditions.

It was evident that some leaf-lesion isolates sporulate while others do not. One isolate obtained from Dr. J. H. Graham at the Northeast Regional Pasture Laboratory produced more conidia on two types of media than most of the New Hampshire isolates. This suggests that genetic factors may control sporulation of this organism. It is obvious that additional studies are needed to improve sporulation.

Some difficulties were encountered in isolation of single ascospores, but at least 30 single ascospore isolates were obtained. However, very few of them produced sclerotia or perithecia in comparison with leaf-lesion isolates. Also, none of them sporulated when grown alone or in mixtures on either agar or grain, under various environmental conditions. No explanation can be given for these results. They are contrary to the results of Chamberlain and Allison (3) who obtained ascospores from single spore isolates, and reported that the fungus was homothallic.

CONCLUSIONS

Conidial formation was very poor among 257 leaf-lesion isolates of P. bromi. A few isolates produced conidia sparsely on PDA at room conditions.

Various media and light treatments did not significantly increase conidial production, although slight increases were obtained on "V-8" agar, when grown at 12° C for 10 days, then at room conditions for 5 days.

Large differences were found between leaf-lesion isolates in rate of growth, colony type, and size and number of sclerotia.

Approximately half of the leaf-lesion isolates grown on a grain medium produced mature ascospores.

Single, leaf-lesion isolates were as effective as a mixture of isolates in production of ascospores on grain.

Attempts to shorten the time required for ascospore production were unsuccessful.

Most single ascospore isolates did not produce sclerotia or perithecia, and none of them sporulated on either agar or grain.

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DEPARTMENT OF AGRONOMY, UNIVERSITY OF NEW HAMPSHIRE, DURHAM, NEW HAMPSHIRE

WOOD PITTING OF UNDETERMINED CAUSE IN UNBUDDED CITRUS SEEDLINGS

J. B. Carpenter and J. R. Furr[1]

Summary

Observations were made on wood pitting of undetermined cause in unbudded citrus seedlings in California. The plants with wood pitting included hybrid and nucellar seedlings of mandarins, tangelos, tangors, oranges, shaddocks, and grapefuit and nucellar seedlings of 'Palestine' sweet lime. Such pitting might cause confusion or errors in experimental work because of its similarity to wood pitting associated with virus infections.

Observations were made from January 1958 to October 1960 on wood pitting in 3- to 7-year-old unbudded seedling citrus trees. The cause of the wood pitting has not yet been determined, but these observations may aid others in investigations of the causes of wood pitting and may suggest precautions that should be taken in some types of experimental work to avoid confusion between inherent and induced wood pitting.

MATERIALS AND METHODS

Approximately 10,000 hybrid and nucellar citrus seedlings have been assembled at the United States Department of Agriculture Southwestern Irrigation Field Station, Brawley, California, through successive field plantings begun in 1953. The trees were planted in the field as unbudded greenhouse-grown seedlings at a 3-foot spacing in rows 10 feet apart. Old citrus orchards exist in the area but not immediately adjacent to the experimental plantings. The large seedling population provides material for a study of the occurrence, incidence, and cause of wood pitting in individual plants and seedling families and for observations on the apparent effects of wood pitting on the usefulness of affected seedlings. Bark and wood specimens were obtained from 136 trees that were marked for destruction because of unsatisfactory fruit characters; 18 trees were retained for further study and the rest destroyed. The trees included nucellar mandarins (Citrus reticulata) and various hybrids involving mandarins, oranges (C. sinensis), grapefruit (C. paradisi), shaddocks (C. grandis), tangors (C. reticulata x C. sinensis), and tangelos (C. paradisi x C. reticulata). The trees were examined for evidence of external and internal abnormalities of the bark and wood of the lower trunk. From 5 to 15 square inches of bark was removed to expose the cambial faces of the bark and wood on seedling trees 3 to 5 inches in diameter near the ground line.

OBSERVATIONS

Of the 136 trees examined, 76 were free of external defects and abnormalities of the cambial faces, and 60 had some degree of wood pitting, with corresponding pegs on the cambial face of the bark (Fig. 1). The pits conformed to DuCharme and Knorr's (3) classes of conoid and deltoid pits and to Winocour's (6) description of conoid pits. Mild fluting (3) was present in some specimens. Gum, when present, was localized in the base of the pegs on the bark. None of the fresh or preserved bark specimens showed macroscopic or microscopic evidence of general gum impregnation. No bark pitting of the types reviewed by Nour-Eldin and Childs (5) occurred. The external bark faces were free of shelling, scaling, and scurfiness; no scale insects or other bark-feeding insects were found in this planting. Two trees showed external grooves distinct from the elongated channels on typical furrowed stems, as well as severe wood pitting in the portions underlying the grooves. One, a tangelo hybrid ('Clement' x 'Orlando'), had deep, narrow external furrows; the other, a mandarin hybrid ('King' x 'Clementine'), had pockets of gum in the wood underlying the grooves.

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Grateful acknowledgment is made to W. W. Armstrong, Jr., formerly Horticulturist in this Division, for photographic work.

FIGURE 1. Bark from lower trunk of hybrid mandarin seedling ('Clementine' x 'Frua') with pegs and mild fluting on the bark.

The parentages of seedling families at Brawley that yielded one or more trees with wood pitting were as follows:

'Clement' tangelo x 'Sunshine' tangelo
'Clementine' mandarin x 'Frua' mandarin
do. x 'Honey' mandarin
do. x 'Ponkan' mandarin
do. x 'Hamlin' orange
do. x 'Ruddy' tangor
do. x 'Minneola' tangelo
do. x 'Seminole' tangelo
do. x 'Sunshine' tangelo
do. x 'Kao Panne' shaddock
'Honey' mandarin x 'Minneola' tangelo
do. x 'Orlando' tangelo
do. x 'Pearl' tangelo
'King' mandarin x 'Batangas' mandarin
do. x 'Clementine' mandarin
Red grapefuit x 'Redblush' grapefruit
'Temple' orange x 'Batangas' mandarin
do. x 'Clementine' mandarin
do. x 'Frua' mandarin
do. x 'Kinnow' mandarin
do. x 'Swatow' mandarin
do. x 'Hamlin' orange
do. x Oasis orange
do. x 'Pineapple' orange
do. x 'Viciedo' orange
do. x 'Minneola' tangelo
do. x 'Orlando' tangelo
'Umatilla' tangor x 'Orlando' tangelo
do. x 'Temple' orange
'King' mandarin, nucellar seedlings

The female parent trees of the hybrid and nucellar seedlings listed had some wood pitting when examined in 1958 and 1960. None of the parent budded trees, however, had more than a few small to medium-sized pegs on the four or more square inches of bark examined. Some faint fluting (3) was observed on the cambial faces of both wood and bark in a few of the parent trees.

Wood pitting of the conoid type (3) was found in 1958 in six unbudded 3-year-old nucellar seedlings of 'Palestine' sweet lime at the United States Date Field Station, Indio, California; by 1960 wood pitting, as indicated by size and number of pits, had increased in these seedlings. No general gum impregnation of the cachexoid type occurred in these seedlings. Some 3-year-old budlings of these seedlings on Rough lemon rootstock also showed mild wood pitting. The seedlings were derived from an old tree at Yuma, Arizona that showed severe wood pitting. When 3 years old, budlings from the parent tree showed wood pitting at Indio. Wood pitting of sweet lime seedlings, resulting from seed transmission of xyloporosis, was reported by Childs (2).

EXPERIMENTS

Investigations now underway at Indio and Brawley on wood pitting in unbudded seedlings include: (a) studies on open-pollinated seedling populations and vegetative propagations of seedlings affected with wood pitting to learn whether wood pitting is of genetic origin, results from some external injury, or represents seed transmission of a virus, and (b) routine virus-indexing studies for identification of known viruses that may be associated with wood pitting.

DISCUSSION

What is the significance of wood pitting in unbudded citrus plants? Does it represent: (a) an accident of growth of no pathological, physiological, or genetic importance; (b) a symptom of infection by a seed-transmitted virus, a possibility indicated by Childs (2) and Norman, et al. (4) in their respective studies on xyloporosis; (c) a genetic character, as suggested by the investigations of Calavan (1) on wood pocket of lemons and limes; (d) infection by an insect-transmitted virus other than tristeza, as DuCharme and Knorr (3) hypothesized; or (e) insect damage and false pitting caused by scale insects, as reported by DuCharme and Knorr (3) in their discussion of vascular pits and pegs associated with diseases? The last possibility is probably not applicable in this study because no scale insects were observed at any time on the plants under consideration and no other bark-feeding insects are known in the area. Is wood pitting, regardless of the cause, harmful to the growth, yield, and longevity of citrus plants? These questions suggested themselves when wood pitting was observed in unbudded citrus seedlings, and answers are being sought.

Meanwhile, the observations reported herein and those of Childs (2) and Norman, et al. (4) suggest that as a precautionary measure in investigations involving citrus rootstock and virus-disease experiments, it may be desirable to grow concurrently with the experimental materials several seedlings of each lot of a given rootstock variety to aid in the interpretation of stock-scion relations as the experiments progress. Furthermore, trees used as seed sources in citrus investigations should be selected with care and indexed for identifiable viruses. The presence of wood pitting in 'Palestine' sweet lime seedlings raises doubts as to its suitability as an indicator plant for xyloporosis; perhaps another indicator is needed for the wood-pitting type of xyloporosis, without generalized gum formation, as distinguished from the cachexia type.

Wood pitting of the unbudded seedlings considered here has not caused any apparent reduction in growth and vigor.

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RELATIVE RESISTANCE OF JUGLANS REGIA SEEDLINGS TO INFECTION BY VERTICILLIUM ALBO-ATRUM AS DETERMINED BY INOCULATIONS[1]

P. W. Miller[2]

Many Persian walnut trees (Juglans regia) in Oregon are in the process of decline (3). Black-line, a disorder of grafted trees characterized by girdling, is usually the cause, but in a number of instances the cause of decline is obscure (3). Numerous fibrous roots of many such declining trees are dead, but the larger lateral roots and crowns are not affected in most instances. Seemingly, decline in such cases is associated with reduction in the functional root area.

In a search for a possible cause of walnut tree decline, the possible role of Verticillium albo-atrum, a widely distributed pathogen that causes decline and death of peppermint (2) and many other plants in Oregon, was considered. A survey of the literature reveals only one report, from France, of the infection of J. regia by a Verticillium species (1).

The present paper reports the results of an attempt to infect J. regia seedlings artificially with V. albo-atrum.

MATERIALS AND METHODS

The roots of ten 1-year-old J. regia seedlings were dipped in a heavy spore suspension, and five seedlings in a suspension of microsclerotia of V. albo-atrum (isolated from an infected maple tree), and planted in sterile soil in 8-inch pots and held from 4 to 12 months in a screenhouse at outdoor temperatures; the soil temperatures ranged from 50° to 80° F. Ten uninoculated potted J. regia seedlings in sterile soil held under the same conditions served as controls. At the termination of the treatment periods, the roots of the inoculated and uninoculated trees were washed free of soil and examined for evidence of infection. Attempts were made to isolate V. albo-atrum from (a) roots and (b) crown and trunk of each of the treated trees.

RESULTS

No evidence of infection by the Verticillium fungus was found in any of the inoculated trees. While a number of small fibrous roots were dead at the end of 4 to 12 months, neither the large fleshy tap root nor the trunk of any of the treated trees showed any evidence of infection. All attempts made to isolate V. albo-atrum from the dead rootlets and trunks of the inoculated trees were uniformly negative. J. regia seedlings apparently are not susceptible to infection by the race of V. albo-atrum used in these tests.

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OBSERVATIONS ON STUBBORN AND OTHER DISEASES OF CITRUS IN MOROCCO IN 1959[1]

J. F. L. Childs[2] and J. B. Carpenter[3]

INTRODUCTION

Symptoms of a citrus disorder variously known as stubborn disease, little leaf, and acorn disease, as well as by other names, have been recognized in Morocco since 1949 (4). Because of the controversial nature of the disorder, its symptoms and its cause, the Moroccan government asked the International Cooperation Administration of the United States Department of State for assistance in determining the nature and extent of the problem. As a result the authors were sent to Morocco to study the stubborn disease problem and from November 18 to December 5, 1950 examined citrus trees in all stages of the disease in six of the seven principal citrus-growing districts of Morocco.

Before reporting the authors' observations on citrus diseases in Morocco, and as an introduction to them, it seems desirable to present a short history of the disorder now generally called stubborn disease, and an outline of the symptoms, and to describe briefly citrus culture in Morocco.

A Brief History of Stubborn

In 1930 Reichert (14) and later Reichert and Perlberger (17) described a disease of budded sweet orange (Citrus sinensis) trees in Palestine that they called "little leaf[4]." The "little leaf" symptoms reported by Reichert were small leaves, multiple buds, short internodes, off-season bloom, stunted growth habit, and misshapen fruit.

In 1944 Fawcett, et al. (10) reported a disease of the same symptoms on sweet orange trees in California. It was termed "stubborn disease" because the stunted and unproductive trees did not respond to corrective treatment (7, 8, 9), such as top-working with budwood from healthy-appearing, productive trees. In fact, the new tops developed symptoms identical with those exhibited by the removed old top. The abnormal shape of some of the fruits, described as acorn shape (10), was a notable symptom of the stubborn trees.

About 1949 Chapot noticed these symptoms on sweet orange trees in Morocco. His identification of the disease was confirmed by A. A. Bitancourt, L. J. Klotz, and J. F. L. Childs, who visited North Africa at various times between 1949 and 1957. In 1956 Chapot found typical symptoms of stubborn, particularly fruit symptoms in Algeria, Syria, Lebanon, and Turkey (3, 4). One of the fruit symptoms noticed by Chapot, and previously observed in Morocco, is best described as "stylar-end greening" or "color inversion" (4); a color transparency of this symptom was sent to Childs in 1956. At the time of the 1957 Citrus Virus Conference at Riverside, California, stylar-end greening was observed on a stubborn Trovita orange[5] tree in Coachella Valley, California and Chapot showed color transparencies of this symptom taken in the Mediterranean area. At that time Chapot (3, 4) and Carpenter (1) reported studies that emphasize the similarity of stubborn disease in Morocco, other Mediterranean countries, and the United States.

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[1]The hospitality of government officials and citrus growers in Morocco is gratefully acknowledged, and appreciation is expressed to the following persons for their assistance and cooperation in expediting the survey: Dr. N. El Ghorfi, Chief of the Agronomic Research Service of Morocco; Mr. H. Chapot, Chief of the Citrus Section, Agronomic Research Service of Morocco; Mr. H. Brent, Chief of the U. S. O. Mission to Morocco; Mr. G. Whitman and Mr. J. P. Emerson of the Food and Agriculture Section, U. S. O. M., Morocco; Mr. H. Watenpaugh of the Soils Section of U. S. O. M., Morocco, and Dr. L. J. Klotz, University of California, Riverside, California. Special recognition is due Dr. J. Bove of the Institut Francais de Recherches Fruitieres, for his superb translation of technical discussions.

[2]Orlando, Florida.

[3]Indio, California.

[4]Reichert afterwards considered "little leaf" disease synonymous with xyloporosis (15) and elected to use the latter name for both disorders. Thus the name xyloporosis may have more than one meaning, depending on the observer.

[5]Trovita is a seedling of Washington Navel orange, first grown at Riverside, California Citrus Experiment Station in 1915 (11).

Published evidence that stubborn is a virus disease consists of an experiment reported by Fawcett (7) and by Fawcett, et al. (10) in which apparently stubborn-free budwood developed typical symptoms when worked on 14 infected trees.

Citrus Culture in Morocco

The visit to Morocco was timed to coincide with the harvest of Washington Navel oranges, because stubborn disease symptoms are most readily recognized in that variety. Traveling by automobile, the survey party[6] visited commercial citrus orchards in the vicinity of the following towns: Rabat, Casablanca, Mechra Bel Ksiri, Sidi Slimane, Sidi Kacem, Meknes, Fes, Beni Mellal, Marrakech, Agadir, and Taroudant, and the experimental plantings at experiment stations near Rabat, Sidi Slimane, Ain Taoujdat, and Marrakech. The citrus area of Oujda in northeastern Morocoo was the only one of appreciable size not visited.

Citrus orchards in Morocco are on irrigated alluvial soils ranging from sandy loams to loamy clays and sour orange (C. aurantium) is the predominant rootstock. Cultural practices are rather similar throughout Morocco. In the older plantings trees were spaced about 23 x 23 feet, but many recent plantings are closer (20 x 23, 30 x 20, and 10 1/2 x 23 feet). Average production is 8900 pounds/acre, but well-managed orchards may produce 13,000 to 18,000 pounds/acre and a few orchards produce 26,000 to 36,000 pounds/acre (4). The 1959-60 citrus crop for Morocco is estimated as 10,144,000 90-pound boxes[7]. For comparison, Florida's average per-acre production of oranges is approximately 32,400 pounds/acre, although a few orchards produce 80,000 pounds/acre.

The principal varieties of citrus grown in Morocco are as follows: Washington Navel and Thomson Navel (early oranges); Hamlin, Cadenera, common (seedy), and Washington (grosse) Sanguine (mid-season oranges); Valencia late, Vernia (late oranges), total 106,253 acres; Clementine, Wilking and Willow Leaf (mandarins) total 10,873 acres; grapefruit, principally Marsh, 3953 acres; and lemons, Eureka and others, 2471 acres. This amounts to 123,550 acres, 74,130 acres of which are producing fruit (1959 figures)[7].

OBSERVATIONS ON STUBBORN DISEASE IN MOROCCO

Examination of stubborn trees of as many varieties of citrus as possible and in as many locations and environments as time allowed was the main purpose of the trip to Morocco. Other diseases were observed however, and are discussed briefly later.

Symptomatology of Stubborn

The symptomatology of stubborn disease is confused and citrus virologists are now wholly agreed on the diagnostic significance of the various associated abnormalities. Consequently it seems desirable to outline the abnormalities on which citrus trees were judged to be affected with stubborn disease in Morocco.

Tree Shape: Trees are stunted, sometimes flat-topped, and twigs commonly exhibit short internodes, giving a dense or "bushy" appearance (Fig. 1) to the tree.

Leaves: Leaves are commonly small, cupped or boat-shaped, with rounded tip, and are often observed standing upright, with dry or leathery appearance (Fig. 1).

Bloom: A few out-of-season blooms are usually present in the fall and winter.

Fruits: Among the normal-appearing fruits there are few to many very small fruits, a few acorn-shaped fruits (thick rind at stem end, thin rind at stylar end, Fig. 2), sometimes others with rind malformed, thicker on one side of the fruit, and occasional fruits with curved columella (Fig. 3). Navels of navel oranges are closed more or less completely, taste is sometimes abnormal to bitter, and stylar-end greening (reversal of the normal order of color development in ripening fruit (Fig. 4) such that the stylar end remains green after the stem end of

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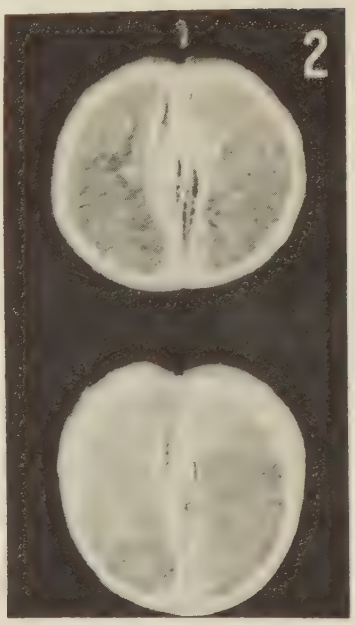
[6]Members of the party were Mr. H. Chapot and Mr. J. Cassin of the Citrus Section, Agronomic Research Service; Mr. Paul Rieuf, Pathology Laboratory, Agronomic Research Service, working in cooperation with the Moroccan government; Mr. J. Bove, Laboratory of Biochemistry, Institut Francais de Recherches Fruitieres, Paris (France); Mr. G. Morel, Laboratory of Plant Physiology, Institut National de la Recherche Agronomique, Versailles (France); Dr. G. Ruggieri, Director of Citrus Experiment Station, Acireale, Sicily (Italy); and Mr. S. Ghazili, Citrus Experiment Station of Tripoli (Lebanon). The group was joined from time to time by government officials and growers.

[7]Data from Citrus Growers Association of Morocco, by letter from H. Chapot.



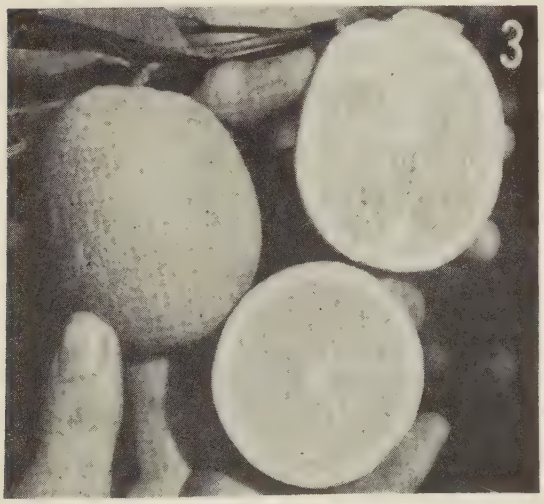


FIGURE 1. Washington Navel orange tree on sour orange rootstock, 17 years old, and severely stunted. It has all the symptoms associated with stubborn disease.

FIGURE 2. Normal sweet orange fruit above and an acorn-shaped fruit below, in longitudinal section.

FIGURE 3. Lopsided fruits; note the curved columella of the one on the right.

FIGURE 4. Washington (grosse) Sanguine, sweet orange fruits; note the green color at the stylar end of the three fruits on the right.

the fruit has become orange colored) occurs instead of normal ripening. Stylar-end greening is associated with stubborn disease in Morocco, but proof that it is a valid symptom of stubborn has not been established. Acorn-shape of the fruit is the only symptom universally accepted as a valid indication of infection by the stubborn virus. Blue (blue-gray) albedo has been described as a symptom of stubborn, but the occurrence of this symptom is erratic and is influenced by certain chemical treatments (1), so that evaluation of its significance is difficult at this time. In some Moroccan orchards relatively large numbers of small fruit with blue albedo were found.

Severe Symptoms: A citrus tree severely affected by stubborn virus is stunted and dense (bushy), owing to the short internodes. The leaves are small, cupped, rounded at the end, and leathery-appearing, and tend to stand upright. Out-of-season blooms are typically present but are not always conspicuous. Many fruits are small and some may be acorn-shaped or malformed. Fruit misshapenness varies from tree to tree, year to year, and perhaps with the climate. At the time of "color-break" some fruits become orange colored at the stem end first, leaving the blossom end green (stylar-end greening). "Color breaking" at the stem end is not the result of the position of the fruit on the tree or its orientation, that is, stem-end up or down or horizontal. In time, the green color disappears from the stylar end and then the fruits cannot be distinguished from fruits that colored normally.

Mild Symptoms: A mildy-affected tree is not noticeably stunted, but it may be small for its age; nor is it bushy. Most leaves are normal, but on one or more branches the leaves and fruits may have pronounced symptoms and one or more long, unbranched sprouts may extend above the crown of the tree. The leaves often stand vertically and appear to be sheathing the sprouts. Out-of-season blooming may be present but is not conspicuous. Small fruits or those with blue albedo are usually present, but are not conspicuous. Malformed and acorn fruits may be found on mildly-affected trees, and greening fruits are commonly found on them in season.

Trees Without Obvious Symptoms: Trees without obvious symptoms may be infected with stubborn virus. The "D" tree (Table 1) was selected as being free of stubborn symptoms, but when the fruits were picked, 3% had symptoms indicative of stubborn infection.

Stubborn Symptoms on Citrus Species and Varieties in Morocco

Symptoms of stubborn disease were observed on the following varieties of sweet orange: Washington Navel, Valencia, Thomson Navel, Washington (grosse) Sanguine, Hamlin, Cadenera, Portugaise, Surprise Navel, Beni Selman, and Petit Jaffa (principally on the basis of tree shape, leaf symptoms, and stylar-end greening); very few acorn fruits were found possibly because of the immaturity of most fruits. Symptoms strongly suggestive of stubborn were seen on Marsh grapefruit (C. paradisi). Stylar-end greening only was observed on mandarins (C.

Table 1. Comparison of the yields of normal and diseased fruit from stubborn-infected, 11-year-old Washington Navel orange trees after 7 years of treatment.

Tree designation	Fertilization	Fruit harvested: Type of fruit	Number	Weight (in kilograms)
A. Severe Stubborn	Standard	normal[a]	33	6.5
		stubborn[b]	80	15.0
		Total	113	21.5
B. Moderate Stubborn	Extra fertilizer	normal	223	31.0
		stubborn	214	28.0
		Total	437	59.0
C. Moderate Stubborn	Extra fertilizer	normal	341	54.0
		stubborn	440	46.0
		Total	781	100.0
D. Healthy appearing	Standard	normal	925	137.5
		stubborn	32	2.5
		Total	957	140.0

[a]Including some fruits too small for marketing by American Standards.
[b]Extremely small and misshapen fruits.

reticulata), Clementine, Willow Leaf, Sanguine, Temple, Sunki, Wilking, and Dancy; on sour orange seedlings; on Kalpi lime (C. aurantifolia) seedlings; and on a citron (C. medica) seedling.

The following species and varieties showed no recognizable fruit symptoms and no foliage symptoms: Lemon (C. limon) variety unknown but Eureka type; and pummelo (C. grandis) variety unknown.

Incidence of Stubborn Disease in Commercial Orchards

Stubborn trees were found in all the citrus areas visited in Morocco. The number of affected trees varied from negligible in some orchards to quite high in others. The short time available permitted few tree-by-tree counts in each area, but the following notes, divested of marginal cases, serve to indicate the situation: In the Mechra Bel Ksiri region, 10% of approximately 7000 Washington Navel trees had symptoms of stubborn; 30% of 12-year-old Valencia orange trees were moderately affected with stubborn; and a few % of 20-year-old Washington Navel trees were affected with stubborn.

In the Sidi Slimane region, 10 to 15% of an orchard of 30-year-old Clementine trees was showing symptoms of stubborn; also 10% of the trees in a Thomson Navel orchard; 80% of the trees in an orchard of Washington Navel, and an estimated 10% of the trees in a Washington (grosse) Sanguine orange orchard were stubborn.

In the Meknes region 38% of the trees of an unknown variety of sweet orange, and most of the trees in a 23-year-old planting of Clementine, had mild stubborn symptoms; many citrus trees on the experiment station grounds at Ain Taoujdat had stubborn symptoms.

In the Beni Mellal region mild stubborn symptoms were present in almost every tree of a 6-year-old planting of Washington Navel orange trees; the same condition existed in a 5-year-old planting of Valencia orange trees; and 10% of the trees in a 10-year-old planting of Washington Navel orange showed severe symptoms of stubborn; many trees in a 10-year-old planting of Hamlin oranges were stubborn from the age of 4 years; and many trees in 17-year-old Valencia plantings exhibited severe stubborn symptoms.

In the Marrakech region, Menara and Souelah Experiment Stations, many trees with stylar-end greening were present in the citrus variety collections.

In the Sous Valley, Taroudant region, 36% of the trees in a 17-year-old Valencia orange planting showed stubborn symptoms; 29% of the trees in a mixed planting of 6- and 12-year-old Valencia orange trees, and 5% of trees in a 17-year-old orchard of Washington Navel orange showed pronounced stubborn symptoms.

Productiveness of Stubborn Trees

Stubborn trees characteristically bear appreciable numbers of fruits as small as 553 fruits per 90-pound box. As the condition of the tree becomes worse and the total number of fruits decreases, the relative number of small fruits increases.

Some information on the effect of stubborn on the production and size of fruit was obtained at the Bonamour farm near Sidi Slimane, as a result of an experiment started there in 1952 by Paul Rieuf, pathologist. In a 10-acre block of Washington Navel trees then 4 years old, three trees showing unmistakable symptoms of stubborn were selected for an experiment. One tree, designated as "A," received the same cultural treatment as the remainder of the block. The other two trees, designated as "B" and "C," were given a "rich additional application" of fertilizer, but the amount was not recorded. After the experiment had been in progress 7 years (Nov. 1959) and after there had been one light picking of fruit, the owner estimated the remaining crop as follows: Tree A -- 10 to 15 kilo, Tree B -- 70 kilo, and Tree C -- 100 kilo. Because Trees B and C were in fairly good condition the owner considered the treatment successful.

At the authors' request, Trees A, B, and C were picked to determine what percentage of the crop was unmarketable because of small size and misshapen condition. For comparison, the crop was harvested from a nearby healthy-appearing tree of the same variety and same age, tree D (two trees distant from tree C). The crop from each tree was sorted by the authors into two categories, normal fruit and stubborn fruit (very small size and/or misshapen). Fruit production on the diseased trees was obviously much less than that of the "healthy" tree D (Table 1). The yield of marketable fruit (normal) from the moderately affected trees (B and C) was approximately 50% of the yield estimated. For example, tree B had 31 kilos of marketable fruit out of 59 picked, and tree C had only 54 kilos marketable out of 100. Obviously tree

D produced more total fruit and several times more marketable fruit than trees A, B, and C. One should not overlook the fact that the trees receiving extra fertilizer (B and C) produced more heavily than the one that did not (tree A produced only 21.5 kilos), but whether the increased production was worth the cost is a question that remains unanswered. Judging by its production of stubborn fruit, tree D is also infected, although mildly.

The effect of stubborn disease on fruit production was observed in another orchard of Washington Navel trees that had shown stubborn symptoms from the outset. At 10 years of age (date of the last picking) they produced approximately 200 boxes per acre. A nearby planting of Hamlin orange trees that showed stubborn symptoms from the fourth year, at 10 years of age produced approximately 350 boxes per acre. The Hamlin trees were twice the size of the Washington Navel trees. Why the Hamlins yielded so much better than Washington Navels is unknown, but it should be noted that the Washington Navels seem to react more severely to stubborn infection than other varieties of sweet orange.

An opinion shared by some observers in Morocco and elsewhere (13, 18) holds that much of what is called stubborn disease is in reality poor growth resulting from bad soil structure, poor drainage, and related causes. However, stubborn trees were not limited to any one area, or soil type, or part of an orchard, but were scattered at random. Alluvial soils or even windlaid soils are not so variable in structure as to account for a poor tree between two healthy ones or vice versa. The soil structure was examined at two locations; in one case, holes approximately 40 inches deep were dug beside a healthy tree and beside a stubborn tree 5 or 6 yards distant. No difference in soil structure was apparent to that depth. At another location the soil structure was observed to a depth of about 5 feet. No hard pan or impervious layers were observed. In both locations the top 9 or 10 inches of soil was darkened by organic matter and graded off into a light brown, loamy clay.

OTHER CAUSES OF POOR CITRUS TREES

Stubborn disease was not responsible for all the unhealthy citrus trees observed in Morocco. Other causes were as follows:

Fungus Diseases: Foot-rot, caused by Phytophthora spp., appeared to be the most important fungus disease of citrus trees in Morocco. It caused considerable damage to lemon trees in several locations, and resulted in some damge to orange trees. Greasy spot, caused by Mycosphaerella horii Hara, was seen on citrus leaves at several locations but was not serious anywhere. Concentric canker caused by Fomes applanatus (Pers.) Wallr. (5), a wound parasite and wood-rotting agent, was seen occasionally on older trees but was not common[8].

Virus Diseases: Next to stubborn disease, the most commonly seen virus disorder of citrus trees in Morocco was the scaly bark form of psorosis. In several orchards it was causing considerable damage. A count of trees with psorosis-A in an orchard of 1100 Valencia trees, 17 years of age, showed approximately 21% infected. Other forms of psorosis, such as concave gum and blind pocket, were much less common.

Xyloporosis (cachexia) symptoms were seen occasionally in the bark of Clementine trees. At present it seems to be causing little trouble in Morocco but would constitute a hazard if susceptible rootstocks such as Rangpur lime or sweet lime were used.

Evidence of exocortis virus was seen only on Rangpur lime in experimental plantings at Ain Taoujdat. At present exocortis is causing no trouble in Morocco but it would prevent the use of Poncirus trifoliata and hybrids of P. trifoliata as rootstocks. Exocortis virus appears to be widely distributed in commercial citrus trees.

Tristeza was found in Meyer lemon in Morocco by H. Chapot and J. Cassin at the time of our visit, but in commercial orchards we saw no evidence of its presence or that it was causing any losses. However, tristeza could be responsible for some of the stunting of sweet orange trees on sour orange rootstock that was seen, and such trees should be indexed on West Indian lime to investigate that possibility. Prof. G. Ruggieri pointed out evidence of Impietratura delle Arance (19), a rind spot that he had experimentally transmitted through budwood. It was seen at several locations, but nowhere was it commercially important. At Ain Taoujdat, bark scaling was noted on Rhobz el Arsa (C. medica x C. aurantium, according to Chapot) rootstock with Clementine mandarin top but the cause is not known.

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[8] A similar disease of citrus trees in Palestine has been attributed to Ganoderma lucidum by Reichert and Avizohar (16).

Deficiency Diseases: Zinc-deficiency symptoms (frenching) were observed on citrus trees in most districts and severe chlorosis was observed in several orchards of the Sous Valley. Heavy applications of nitrogenous fertilizers that caused a greater need for zinc doubtless accentuated the problem. Indications of magnesium and manganese deficiencies were seen occasionally, but usually in conjunction with strong zinc deficiency, which obscured the symptoms and made the diagnosis uncertain. Iron deficiency (lime-induced chlorosis) was rather common, but less so than zinc deficiency.

Diseases of Unknown Cause: Gummosis (Florida gummosis, or Rio Grande gummosis) was observed frequently on lemon trees and occasionally on sweet orange trees but was not causing serious damage.

CONCLUSIONS

Stubborn disease appears to be the most serious disorder of citrus trees in Morocco. Symptoms were observed in all the citrus regions visited and in certain orchards a majority of the trees exhibited symptoms. The manner in which stubborn spreads from tree to tree is not known, and the few experimental transmissions leave many questions unanswered. The occasional appearance of stubborn symptoms on previously healthy trees, 15 years of age and older, suggests that such trees became infected after they were planted and raises the question of whether the disease can be transmitted by insects.

The authors saw no evidence that stubborn symptoms result from poor soil structure, bad drainage, or other soil conditions (13). However, stubborn symptoms often appear to be accentuated by factors that depress tree vigor, such as damage to roots by various causes and inadequate or unbalanced fertilization. Conversely, factors favorable to tree vigor, such as heavy fertilization and small crop, appear to make stubborn symptoms less pronounced.

Other disorders occasionally caused damage to citrus trees in Morocco. Psorosis was generally present and was causing appreciable damage in several orchards. Foot-rot (Phytophthora spp.) was generally present also and causing considerable damage in a few orchards.

Minor-element deficiencies, principally zinc and iron, were serious in certain districts.

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TOBACCO DISEASES IN VENEZUELA[1]

W. D. Valleau

The writer spent the months of March 1959 and February 1960 in Venezuela visiting tobacco fields. The observations seem worth recording as they add to the notes published by Wolf (4)[2].

Flue-cured, burley and Turkish or aromatic tobaccos are grown, as well as some native air-cured tobaccos. Guachero, a small native dark tobacco, is grown by the Canary Islanders around their homes for pipe smoking. This tobacco appears to be an important source of mosaic in commercial plantings.

MOSAIC

This disease was fairly general in Virginia tobacco toward harvest time. It was rare in burley tobacco because nearly all of the burley varieties were resistant (Ky 35, Ky 56, Ky 57, and Ky 58). Burley tobacco plants inoculated with mosaic at pulling or setting died from systemic necrosis during the next 2 or 3 weeks. This was sometimes confused with black shank. Labor taken from a field of Virginia tobacco with mosaic to mosaic resistant burley for the purpose of topping resulted in necrotic streaking from the top down the stalk in the majority of plants. The chief sources of mosaic in Virginia tobacco appeared to be Shimo, a black twist dipped in a sauce made by boiling down tobacco trash, and native tobacco smoked in pipes. Mosaic was transferred from both to tobacco.

ETCH

This virus disease, originally discovered on the Kentucky Agricultural Experiment Station farm at Lexington, had not been recognized or reported in Venezuelan tobacco previous to the 1959 crop, but evidence was obtained that it had been present earlier.

The disease was first seen by the writer in an extensive field of mosaic resistant burley near El Sombrero in 1959. The tobacco was about half-grown. Vegetables (such as pepper, potatoes, tomatoes) were also grown on the farm and in the vicinity. An older, nearly mature crop of burley nearby had comparatively little etch. Both crops had a heavy infestation of winged aphids which did not colonize on the tobacco. A collection of these aphids were later identified as Aphis craccivora Koch. It is well known that the virus is transmitted by aphids, as Kassanis (1) showed that it could be transmitted by six species.

This field was again visited in 1960. There were again two crops of burley, an early one that was about ready for harvest that appeared almost free from etch, and a large planting across the road that was about one-third grown. Etch was just beginning to appear in it. Again there were many winged aphids on nearly every tobacco plant. They were not colonizing. Mr. Lewis Flowers, who had been making observations on etch and aphids for the previous 2 months, pointed out the numerous purple wingless aphids on pigweed, purslane and other weeds and the fact that winged forms were developing in abundance on the weeds. Collections from several weeds, including pigweed (Amaranthus sp.), purslane (Portulaca sp.), a citrus seedling, peppers and potatoes and the winged forms from tobacco all proved to be Aphis craccivora[3]. This aphid was collected from weeds and tobacco in other fields where etch was developing in partly grown tobacco and was collected from the leguminous tree Gliricidia sepium, a tree much used for fence posts, many of which root. This tree remains green during the dry season and carries a heavy population of this aphid but shows no virus symptoms.

Mr. Flowers pointed out virus symptoms on young plants of pigweed that consisted of several alternate chlorotic and green lines more or less paralleling the midvein of the leaf. The patterns were similar to those pictured by McKeen (2) on pepper leaves infected with etch virus in Ontario, Canada.

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[1]The investigation reported is in connection with a project of the Kentucky Agricultural Experiment Station and is published with the approval of the Dean and Director.

[2]The trips were made at the request of Compañia Anonima Cigarrera Bigott Sucs.

[3]Aphid determinations were made by Miss Louise M. Russell, Entomology Research Division, Agricultural Research Service, United States Department of Agriculture.

Etch was seen in flue-cured tobacco where it produced distortion in the growing point leaves similar to that in burley but did not cause conspicuous chlorosis found on the lower leaves of the burley. Etch was not seen on tobacco growing at a distance from vegetable-growing areas.

In 1959 leaves that were well developed when infection with etch occurred showed prominent veinbanding, a symptom with which we are not familiar in Kentucky. This symptom was not seen in 1960 but observations were made a month earlier.

The history of etch development in a field appears to be about as follows:

The field is plowed in the fall toward the end of the rainy season and kept cultivated until the tobacco is set so there are no weeds at that time. The tobacco is irrigated after setting and considerable dirt is moved toward the plants following irrigation, which helps discourage weed growth. The early crops of burley are set in November or early December and these seem to come through relatively free from etch. Crops set in January appear to be those subject to etch. As these crops develop the weed population also increases in the tobacco, and along the irrigation ditches and roadways. Aphis craccivora moves in and becomes established on the seedling weeds, evidently bringing the etch virus in at the same time. It is not until the aphid population builds up on the weeds to a point where winged forms are produced that etch begins to appear in the late-set tobacco. Thorough control of either weeds or aphids within the irrigated area should control the disease, as the native vegetation is nearly all dead or dormant during the dry period and would afford little pasture for aphids and probably no source of the virus.

VEINBANDING

Burley plants affected by veinbanding were found near San Juan de los Morros among plants affected with etch. Potatoes were grown about 200 yards away. In the writer's experience, the veinbanding or potato Y virus occurs only in potatoes and in no perennial solanaceous wild plant.

STREAK

Wolf (4) reported that in Venezuela "In burley fields, streak was found to be present everywhere, but only on occasional widely scattered plants." No streak was found in 1959 or 1960.

LEAF CURL

This virus disease was present both years in all fields examined but caused most loss in early planted fields. Early planted fields sometimes had to be disked and replanted.

Others (5) have described several degrees of severity of what was considered to be the leaf curl disease. In this survey there was a range of symptoms in the fields inspected. The most severe was a rosette condition in which several normal leaves were surmounted by a rosette of small but otherwise normal appearing leaves. Some plants were dwarfed but had severely curled leaves. Others made much more normal growth so far as leaf size was concerned but the large leaves had wavy mid- and lateral veins resulting in the typical curl symptoms. The leaves of curl plants all were mottled yellow and green. Other plants of Virginia tobacco appeared normal, except for yellowish blotches scattered over the leaf surface. Enations were seen on only one plant.

Whiteflies, the vector of curl, were not found colonizing on tobacco either year, in fact they were rare and hard to find. They were found in abundance on Sida rhombifolia, in small numbers on other species of Sida, and on Ipomoea quinquifolia, hosts of the curl virus (5). Tomatoes were frequently affected by curl.

FUSARIUM YELLOWS OR WILT

Fusarium yellows was not seen in Virginia tobacco, which is much more resistant than burley, but was found in several fields of burley. One field of burley along the Guarico River near San Sebastian had 33% of the plants affected.

BLACK SHANK

Black shank was seen near Altagracia de Orituco on Virginia tobacco, near San Juan de los Morros on Turkish tobacco, and near the towns of Guanare, Miranda and Valencia on Virginia tobacco. Loss was small in each field because resistant varieties, usually Dixie Bright 101, were grown. No black shank was seen in burley plantings.

ROOT-KNOT NEMATODES AND SCLEROTIUM BATATICOLA TAUB. OR ASHY MOLD

Root-knot nematodes were widely distributed and causing trouble where tobacco had been grown several years in succession[4]. In these fields at or near harvest time there was frequently heavy infection with Sclerotium bataticola, a cellulose destroying fungus which probably attacks plants the roots of which have been injured severely by nematodes or by overirrigation.

MISCELLANEOUS DISEASES

Frogeye (Cercospora nicotianae Ell. & Ev.) was seen in two Virginia tobacco plantings in 1959, one near Turen and one near Miranda. The spots were large and at first appeared to be brown spot. In 1960 Cercospora was much more common, apparently because the wet season extended into January. Some crops of burley were severely injured. No brown spot was seen on either type of tobacco. Bacterial black stalk (3) was found in a field of Virginia tobacco near Turen in 1959 and a single affected plant of burley was seen near El Sombrero in 1960. One field of Virginia tobacco was seen with lens-shaped cracks on the stalk near the ground which were filled with root tips. The first 6 or 8 leaves showed mild but typical 2, 4-D injury. The plants had been sprayed twice with an insecticide using a sprayer that had been used 2 years previously to apply a weed killer. Ring-spot (virus) was not seen either year.

POTASH DEFICIENCY

A serious condition developed in some tobacco fields near Turen, from potash starvation in Virginia tobacco in 1959. The tobacco cured very dark and was nearly valueless. Some plantings were not harvested at all. The worst of this tobacco was grown after sesame. The 1960 crops, in the areas where potash starvation was evident in 1959, were properly fertilized with potash containing fertilizer with satisfactory results.

GREEN PEACH APHIS, MYZUS PERSICAE (SULZ.)

The strain of the green peach aphid that colonizes on tobacco appeared for the first time in noticeable numbers on the 1959 crop of tobacco. Growers and others familiar with the crop stated that they had never seen aphids colonizing on tobacco before. So far there has been no indication of this aphid's playing a part in the spread of etch or any other tobacco virus in Venezuela.

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[4]One collection from burley tobacco growing near Tinaco was identified by Drs. Golden and Taylor, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, as Meloidogyne incognita acrita Chitwood.

A VEIN CLEARING VIRUS OF SWEETPOTATO IN GHANA

G. C. Clerk[1]

Abstract

A hitherto unreported virus disease of sweetpotato, Ipomoea batatas, in Ghana is described. The name Vein Clearing Virus is suggested.

Vines of sweetpotato growing on peasant farms at Tafo in the Akim Abuakwa District were observed to be showing apparently virus-like leaf symptoms accompanied by marked stunted growth. This has now been confirmed experimentally to be a virus disease and hence hereafter referred to as Vein Clearing Virus after the nature of the symptoms.

All attempts to transmit the virus into healthy shoots of sweetpotato by mechanical inoculation (using expressed infected sap and celite; infected sap with celite buffered by phosphate) have proved unsuccessful. Grafting (Patch grafting) however has been exceedingly successful, giving approximately 86% infection in this preliminary work. Of the 60 healthy stocks grafted on March 21 with infected scions, 52 showed the symptoms. The first symptoms appeared on May 2, 42 days after grafting. The virus was readily transmitted by tuber core-grafting. Dodder, Cuscuta chinensis, failed to transmit the virus. Dodder could not establish itself on sweetpotato in all the trials made.

The principal vector was found to be the whitefly, Bemisia tabaci. Whiteflies collected from sweetpotato in the field were released on May 16 into insectaries containing 30 infected and 30 small healthy rooted cuttings. Clear symptoms were first observed after 5 to 6 weeks. On July 30 (end of the tenth week of the experiment) when the final count was taken, 9 out of the 30 healthy stocks were diseased. No invaders were detected throughout this period.

The most obvious and usual symptoms are vein clearing, reduced leaf area, shortened internodes, and a definite stunting of the plant. In varieties with dissected leaves, segments may be strap-shaped (Figs. 1A, 2A, and 3).

In many plants the primary symptoms on infection consist of small bright yellow spots which occur in random fashion over the leaf. These are mainly attached to the veinlets. With the progress of infection the specks concentrate along the main veins, finally bringing about a clearing of the veins. On other plants, the yellowing of the veins is accompanied by development of chlorotic areas of varying width along the main veins (Fig. 4 and middle leaf of Fig. 2A). Symptoms of both types, however, never appear after the plant has been infected for some time, when there is then a consistent reproduction of vein clearing symptoms only.

Local information points out that the disease was observed some years earlier in the area, and the remarkable extent of the disease seems to confirm it. Obviously, spread of the disease has been encouraged, rather unwittingly, by peasant farmers in establishing new farms with diseased stem cuttings.

The occurrence of virus diseases in sweetpotato has been reported by various workers from other parts of the world; the first record of a disease in this host seems to be that of Ensign (3). Rosen (9) reported a mosaic disease in Arkansas, and in Florida Weber (14) noted a mosaic that caused a stunting and rosette-like appearance of the affected plants. In a later paper Weber and West (15) reported what they considered to be a second type of mosaic on sweetpotato in Florida. Doolittle and Harter (2) described the feathery mottle disease in the United States. Nusbaum (8) described the internal cork disease in sweetpotato tubers in South Carolina, and Adsuar (1) described a mosaic in Puerto Rico. Mosaic mottle is a severe disease in Ceylon (7). In the Ryukyu Islands, Summers (12) reports "witches' broom" and dwarf which are both quite similar to Thung's "witches' broom" (13). In Africa a suspected virus disease of sweetpotato was reported from Ituri in Belgian Congo in 1939 and then from Uganda in 1944 (4). Similar reports followed within a few years from Kenya, Tanganyika Territory, from parts of Belgian Congo, Ruanda Urundi, Transvaal and Nyasaland. Recently Sheffield (10, 11), reporting on the disease's distribution in parts of East Africa, Belgian Congo and Ruanda Urundi, the host range of the virus and vector relationships, suggested that it has long been established in Africa. She also confirmed that the disease was indeed due to a virus infection, and pointed out that at least two viruses, which she called Virus A and Virus B, were involved.

[1]Plant Pathologist, Ministry of Agriculture, Ghana, W. Africa.

FIGURE 1. A -- Leaves of sweetpotato (dissected leaf variety) infected with Vein Clearing Virus. B -- Healthy control.

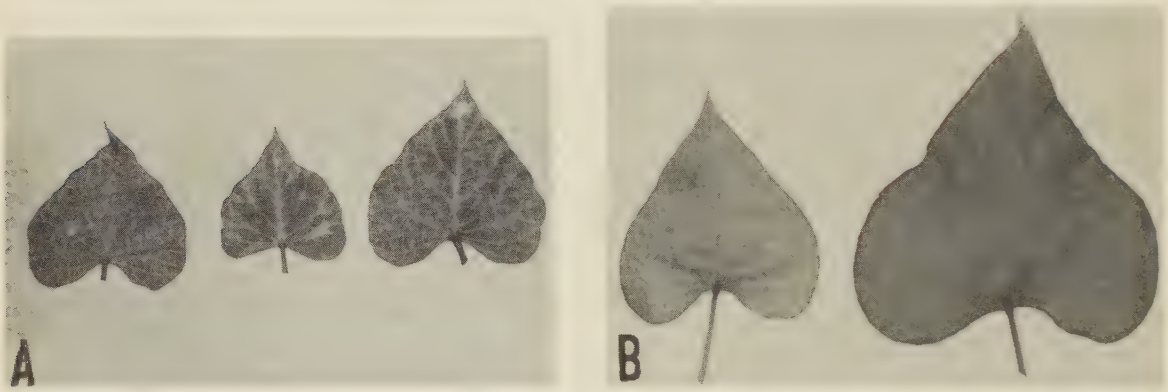

FIGURE 2. A -- Diseased leaves of entire leaf variety. B -- Healthy control.

FIGURE 3. (left) 10-week-old sweetpotato plant (on left) showing characteristic stunting habit of the disease. Healthy control on right. Both were raised from 2-inch apical cuttings from diseased and healthy stocks, respectively.

FIGURE 4. (above) Symptoms occurring in early stages of the disease.

According to Wellman (16) the south celery strain of cucumber mosaic virus occurs naturally in sweetpotatoes in Florida. The last and most recent report is that of Loebenstein and Harpaz (6), who reported three different virus diseases in sweetpotato in Israel.

The type found in Ghana and being studied at the moment resembles superficially that described by Sheffield which she called Virus B, and the Vein Clearing Virus of Israel. Even though there is some degree of similarity between the two diseases of East Africa and Israel, Sheffield reports cork symptoms associated with the Virus B, whereas these symptoms were never noticed in the disease of Israel. Moreover, Virus B happens to be sap-transmissible to other hosts, while the Vein Clearing Virus of Israel is not. On the strength of these, the Vein Clearing Virus of Israel seems to be quite different from Virus B of East Africa. Work which is being conducted on the Ghana disease may reveal any relation or none between Ghana's Vein Clearing Virus and any of the viruses of East Africa and Israel.

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METHODS OF CONTROLLING CERTAIN DISEASES OF SHALLOTS

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Abstract

Treating diseased shallot bulbs with Dowicide B before planting resulted in increased yields. Soil treatments with PCNB were also often beneficial if conditions favored the development of Sclerotium rolfsii. Drying bulbs in the field after harvest resulted in less loss in storage than when bulbs were brought in to storage immediately, even when they were dried artificially for 6 days in heat chambers. The use of Dowicide B on dried bulbs also reduced the amount of black mold (Aspergillus niger) and improved the appearance of the bulbs.

Shallots (Allium ascalonicum) are grown only to a limited extent in North Carolina, although the Wilmington variety which was introduced in 1954 (4) is well adapted and produces high yields of both green shallots and mature bulbs. The failure of growers to increase acreages may be attributed in part to serious production problems resulting from losses by diseases that attack both the growing plants and the mature bulbs. Heavy losses occur in both field and in storage.

Important field diseases are caused by Botrytis allii Munn and Sclerotium rolfsii Sacc. Bacterial soft rots and Fusarium bulb rots are also often noted. The storing of bulbs infected with these organisms results in further losses. Since 1956 the effectiveness of certain bulb and soil treatments and postharvest handling practices in reducing disease losses have been studied and are reported here.

REVIEW OF LITERATURE

Walker (7, 8, 9) described three species of Botrytis which cause a neck rot disease of onions. Descriptive names were given to the diseases caused by each species as follows: gray-mold neck rot (B. allii Munn); small sclerotial neck rot (B. squamosa Walker) and mycelial neck rot (B. byssoidea Walker).

Gray-mold neck rot, the most common of the three diseases, is usually found on the bulbs after harvest. Infection occurs through the neck tissue or through wounds elsewhere. The disease proceeds down the scales rather rapidly but more slowly through the bulbs horizontally. A mycelial mat develops under the scales with a profuse production of conidia, resulting in the characteristic gray mold appearance. In the terminal phase of the disease whitish sclerotia are formed which are irregular in shape and turn dark with age. Although B. allii is primarily a parasite of dormant fleshy scales, it sometimes causes small white necrotic lesions on leaves and seed stalks.

Heavy losses from Sclerotium rolfsii were encountered soon after the introduction of shallots in southeastern North Carolina (3). Tims (6) recently reported that S. rolfsii occasionally occurs in late season on shallots in Louisiana, but that in 1955 it became serious in April and May near Baton Rouge. It also caused slight losses in commercial fields in June in other areas of Louisiana. The softening and general disintegration of the outer scales of infected bulbs that he described is typical of the symptoms observed in North Carolina. Sclerotia develop on the tissue and surrounding soil following death of the plants.

The only disease control practices for shallots that have been recommended in North Carolina are crop rotation and the use of disease-free planting stock (3). Drying of harvested bulbs in the field followed by storage in thin layers in well ventilated buildings has been suggested in Louisiana and North Carolina (3, 6).

Artificial curing of onion bulbs after harvest by heating in dry air currents at 37° to 48°C has been recommended for the control of neck rot of onion, but according to Walker this has been used in only a limited way commercially. A temperature of 0°C or just above with a relative humidity of 65% is recommended for table-stock onions and onion sets (10).

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METHODS

Bulbs of the Wilmington variety were used in all experiments. They were grown on sandy loam soils. Approximately 500 pounds of 5-10-5 was mixed into the soil before planting followed by a like amount as a side-dressing when the plants were 8 to 10 inches high. Unless otherwise indicated all plantings were made in September. A uniform number or weight of bulbs was used for each plot in individual experiments but this amount varied among experiments. Plot size was usually two rows 50 or 100 feet long. Bulbs were harvested in May and stored in shallow, wire-bottomed bulb trays.

EXPERIMENTAL PROCEDURE

Bulb Treatments: In North Carolina B. allii occurs on shallots in the field during the winter months. The fungus causes a soft decay of the stem. Although infection usually occurs at ground level or above, it may proceed to some extent down the scales below ground. Sporulation is profuse on the scales above ground and between the newly forming bulbs below ground. The roots are unaffected.

During November and December temperatures frequently drop below the freezing point in this area. The young leaves of recent transplants are often injured or killed during such periods, and these leaves are frequently covered with myriads of Botrytis spores. As has been pointed out by Walker (10), the three species of Botrytis which parasitize onions grow and reproduce over a wide temperature range, but infection and decay of bulbs are clearly favored by temperatures of 15° to 20°C. Such conditions are possible for a number of hours daily for extended periods in southeastern North Carolina during the winter months. Botrytis has not caused serious losses in shallots grown from bulbs planted in September which were not divided and reset in mid-winter.

Because commercial gladiolus growers have been very successful in controlling storage rots and field diseases (caused principally by Fusarium and Botrytis) with corm dips (1), a number of experiments involving the use of pre-planting dips were conducted with shallots over a 3-year period.

Three materials, sodium 2, 4, 5-trichlorophenoxide (Dowicide B), N-trichloromethylmercapto-4-cyclohexene-1, 2-dicarboximide (captan) (Orthocide 75) and zinc ethylene bisdithiocarbamate (zineb) (Dithane Z-78) were tested using three sources of bulbs in 1955-56. Two of these lots were selected from reasonably clean bulbs grown at the Horticultural Crops Research Station the previous year. They were both planted in September, but in different locations. No increases in yield over the control were obtained by the use of these preplanting treatments on these two lots. The third lot, obtained from a grower who had sustained serious losses due to Botrytis in 1955, was planted in January. Significant increases in yield as a result of the use of fungicidal dips were obtained only with the Botrytis-infected bulbs. Results are shown in Table 1. Differences in vigor of plants were obvious in the field. Both rates of Dowicide B and captan at 12 pounds/100 gallons resulted in increases of more than 50% over the control.

Table 1. Effect of preplanting bulb treatments on yield of shallots.

Treatment and rate (pounds/100 gallons)	Time (minutes)	Yield (in grams)
Dowicide B 1 3/4	25	1815
Dowicide B 3	15	1731
Captan 12	25	1868
Zineb 2	25	1175
Control	-	1076
LSD .05		525

Soil and Bulb Treatments: The wide host range of Sclerotium rolfsii, the large number of susceptible vegetable and ornamental crops grown in the Wilmington area, and the warm soil temperatures in the spring results in serious losses from this organism in certain seasons. A high incidence of this disease has been noted as early as mid-April.

Losses in North Carolina under some conditions have approximated 50% although usually they are much less. Often bulbs in the early stages of infection continue to rot severely in stor-

age particularly when they are not allowed to dry down or are stored in deep layers. Other decays apparently caused by Fusarium, bacteria, and undetermined agents are associated with or follow S. rolfsii.

Because of the importance of Sclerotium rolfsii as a soil-borne pathogen, preplanting dips were compared alone and in combination with pentachloronitrobenzene (PCNB) (Terraclor) soil treatments. PCNB, 10% granular, was applied at the rate of 50 pounds active ingredient/acre in two applications. Twenty-five pounds was distributed into furrows and stirred into the soil before bedding and planting. The remainder was distributed on either side of the plants between the first and fifteenth of April each year.

In 1957 bulb treatment (Table 2) had no significant effect either on the incidence of S. rolfsii at harvest time, or on the yield. Highly significant effects were noted both in reduction of S. rolfsii and in increases in yield where PCNB soil treatments were used. These increases amounted on the average to considerably over 50%.

In 1958-59 the incidence of S. rolfsii was lower where bulbs received a preplanting dip with PCNB but these differences were not significant (Table 3). PCNB soil treatments again

Table 2. Effect of soil treatment on incidence of Sclerotium rolfsii and yield of shallots[a].

Soil treatment and rate	% of clones showing signs of S. rolfsii at harvest	Net gain[b] (in grams)
PCNB 50 pounds/acre[c]	12.0	362
None	21.8	207
LSD .05	5.0	111
LSD .01	6.8	152

[a] Means of preplanting bulb treatments which included maneb (manganese ethylene bisdithiocarbamate) (Manzate, 12 pounds/100 gallons and 2 pounds/100 gallons); captan (12 pounds/100 gallons); Dowicide B (1 3/4 pounds/100 gallons and 3 pounds/100 gallons); methylmercury dicyandiamide (Panogen, 3 1/2 pints/100 gallons); PCNB (2 pounds/100 gallons) and the control were not significant. Bulb treatment means included PCNB and no soil treatment.

[b] Increase in weight of harvested bulbs over those planted.

[c] Applied in split applications; 25 pounds at planting, 25 pounds in April.

Table 3. Effect of preplanting bulb and soil treatments on incidence of Sclerotium rolfsii and yield of shallots.

Bulb treatment and rate[a] (pounds/100 gallons)	Time (minutes)	% plants showing signs of S. rolfsii at harvest	Yield[b] (in grams)
PCNB 12	15	9.8	2179
Dowicide B 3	15	17.0	2896
Thimerosal 1/4	15	30.0	2655
Delsan A-D[c] 12	15	18.2	2401
Control	-	24.0	2211
LSD .05		N.S.	343
LSD .01		N.S.	480

[a] Half the bulbs were planted in soil treated with 66 pounds PCNB (75% WP) in two applications of 33 pounds each, one made prior to planting, the other in mid-April. The remainder of the bulbs was planted in non-treated soil. Incidence of S. rolfsii in the non-treated plots was significantly higher than the untreated but yields were not.

[b] From 100 linear feet of row.

[c] Active ingredients: thiram 60% (tetramethylthiuram disulfide), dieldrin 15% (1, 2, 3, 4, 10, 10-hexachloro-6, 7-epoxy-1, 4, 4a, 5, 6, 7, 8, 8a-octahydro-1, 4-endo-exo-5, 8-dimethanonaphthalene).

reduced the number of plants infected at harvest but these differences were not reflected in increased yields. Both Dowicide B and sodium ethyl mercuri thio salicylate[2] preplanting bulb treatments resulted in significant yield increases, however.

Drying and Curing Experiments: Although field drying has been suggested for shallots (3), as has already been noted, actual comparisons of methods of curing and storage had not been made under North Carolina conditions. Accordingly, studies were carried out in which the keeping quality of bulbs dried in direct sunlight along the row in the field for 6 days were compared with those brought in as soon as they were harvested. The dried and freshly dug lots were each further subdivided into six lots and treated as follows: 1) dipped in Dowicide B solution; 2) dipped in Dowicide B solution and then cured 6 days at 100° F; 3) cured at 100° for 6 days and then dipped in Dowicide B solution; 4) cured at 100°; and 5) dried under a fan for 6 days. After treatment all of the lots were placed in common storage in wire bottom trays. Twenty clones made up a single experimental unit and each treatment was replicated four times. Curing at 100° was accomplished by placing the bulbs in closed heat chambers which were ventilated only to the extent that vaporized moisture could escape. Dowicide B solution was used at a concentration of 1 3/4 pounds/100 gallons water. Evaluation of each treatment was made in mid-August.

The bulbs dried in the field behaved as one population, those brought in immediately as another. The mean number and weight of sound bulbs after storage was significantly greater in the former. Moreover, in the field-dried lots no significant differences among sub-treatments were apparent in weight loss in storage or in weight and number of sound bulbs after storage. In the lots brought in immediately, however, real differences among curing and postharvest handling practices occurred. Except for one injurious treatment, all were significantly better than the control. Severe injury resulted from treating freshly harvested bulbs with Dowicide B and subsequently subjecting them to 100° F for 6 days. Considerable breakdown and disintegration resulting in the production of a sticky, foul-smelling substance occurred in the heat chamber. While the three best treatments yielded significantly more than the control, they did not differ significantly from each other in the number or weight of sound bulbs present after storage. Curing at 100° immediately after harvest, however, appeared to be slightly superior to dipping in Dowicide B and storing without heating (Table 4).

Table 4. Effect of various postharvest handling practices on keeping quality of shallots.

Treatment	Weight of sound bulbs after storage (in grams): Field dried[a]	Brought in immediately	Mean (in grams)
Dowicide B, storage[b]	1216	1022	1119
Dowicide B, 100° F, storage[b, c]	1109	643	876
100° F, Dowicide B, storage[b, c]	1191	1133	1162
100° F, storage[c]	1162	1142	1152
Dried under fan, storage	1173	901	1037
Control, storage	1133	555	844
LSD .05	N.S.	215	152
LSD .01	N.S.	289	205
Mean	1164	899	

Means for main treatments were significantly different:
LSD .05 = 115; LSD .01 = 211

[a] Bulbs allowed to dry for 6 days along the row.
[b] Dowicide B solution used for 15 minutes at concentration of 1 3/4 pounds/100 gallons water.
[c] 100° F for 6 days.

[2] This compound has been released under the brand names of Thimerosal and Elcide 73.

Table 5. Effects of various post harvest handling practices on Aspergillus mold index after storage.

Treatment	Mold index[a]		
	Field dried[b]	Brought in immediately	Mean
Dowicide B, storage[c]	1.09	1.39	1.24
Dowicide B, 100° F, storage[c, d]	.78	2.30	1.54
100° F, Dowicide B, storage[c, d]	.63	1.15	.89
100° F, storage[d]	1.45	1.98	1.71
Dried under fan, storage	1.42	1.52	1.47
Control, storage	1.34	1.66	1.50
LSD .05	.64	.64	.45
Mean	1.11	1.66	

Means for main treatments were significantly different: LSD .05 = .47

[a] Based on a 0-4 scale: 0= No Aspergillus; 1= slight infection; 2= moderate infection; 3= moderately severe; 4= severe.

[b] Bulbs allowed to dry for 6 days along the row.

[c] Dowicide B solution used for 15 minutes at concentration of 1 3/4 pounds/100 gallons water.

[d] 100° F for 6 days.

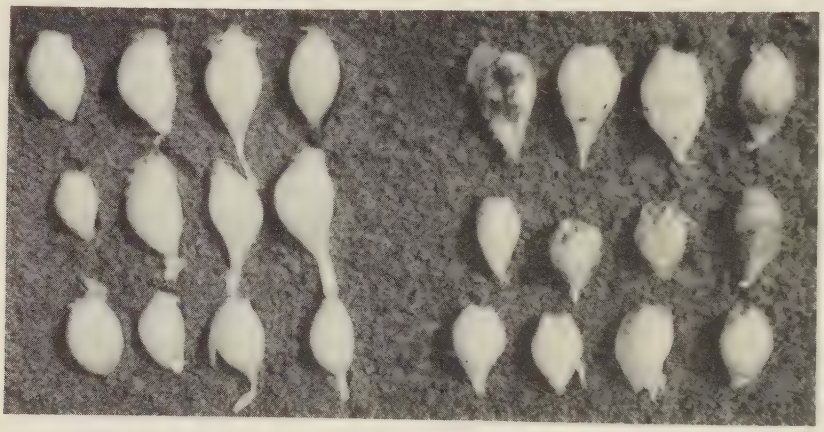

FIGURE 1. Left -- Cured shallot bulbs dipped in Dowicide B solution (1 3/4 pounds/100 gallons for 15 minutes) prior to storage. Right -- Cured bulbs stored without dipping, showing black mold (Aspergillus niger) development.

Only during the first 6 days of curing and storing (when some lots were heated) were there significant differences in percentage weight loss among treatments within the dried lot. Percentage loss varied from 15.9 when bulbs were dipped in Dowicide B and heated at 100° F for 6 days to 6.7 for the control and 7.9 when bulbs received only the prestorage dip with Dowicide B. Among the lots brought in freshly harvested, percentage losses were naturally much greater but they followed the same trend as those among the dried.

During storage considerable black mold (Aspergillus niger van Tiegh.) developed in some lots (Fig. 1). Average ratings determined for each treatment as explained in Table 5 revealed that significantly more black mold was present in those lots brought in from the field immediately. The least amount occurred when bulbs were dried in the field, cured at 100° F for 6 days, dipped in Dowicide B and stored. The greatest amount developed where severe chemical injury occurred, that is, when freshly harvested bulbs were dipped in Dowicide B and cured at 100° 6 days before storing. In the field-dried bulbs Aspergillus was much more noticeable in bulbs which were not treated with Dowicide B, even in those subjected to 100° temperature before storage.

DISCUSSION AND CONCLUSION

Although the role of all the organisms involved in shallot diseases in North Carolina is by no means completely understood, certain practices appear to be beneficial in reducing losses in field and storage.

General observations indicate that Botrytis is usually not a problem except where clones are separated in mid-winter and the single plants re-set for increase. Severity of the disease is increased when these small plants are injured during cold spells.

Treating bulbs before planting has proved beneficial in two seasons out of three. Since Dowicide B used as dip did not result in noticeable phytotoxicity, a preplanting dip would be advisable if planting stock has a history of Botrytis or other troubles. Soil treatments with PCNB for control of Sclerotium rolfsii are likely to be helpful, particularly in seasons with high soil temperatures in April. Certain cultural practices (2) designed to reduce the amount of organic matter in the soil which have proved beneficial for other crops should be adhered to also, in order to avoid severe losses caused by S. rolfsii.

Drying bulbs in the field is the preferred way to handle shallots after digging if clear sunny days follow. If weather conditions are unfavorable, the application of artificial heat to promote rapid drying will reduce storage losses. It is not advisable to dip freshly harvested bulbs in Dowicide B because of the possibility of severe injury, particularly when exposed to high temperatures. The use of Dowicide B or perhaps some other less toxic fungicide after curing may be helpful in cutting down black mold in storage.

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COMPARISON OF FOUR APHID SPECIES AS TRANSMITTERS OF BARLEY YELLOW DWARF VIRUS FROM OAT FIELD SAMPLES IN NEW YORK[1]

W. F. Rochow[2]

Summary

Leaves from 74 spring oat plants collected during 1960 in six New York counties were tested for infection by barley yellow dwarf virus (BYDV) by means of Rhopalosiphum padi, Macrosiphum granarium, Rhopalosiphum maidis, and Toxoptera graminum. The virus was recovered from 64 samples by M. granarium only, from 6 by M. granarium as well as by 1 or more of the other aphid species, from 1 by both R. padi and T. graminum, and from 1 by R. maidis only. During 1957, 1958, 1959, and 1960, BYDV was transmitted specifically by M. granarium from 81, 85, 84, and 86%, respectively, of the 197 spring oats collected in New York and tested during the 4 years by means of both M. granarium and R. padi. Several lines of evidence suggest that M. granarium was the most important natural vector of barley yellow dwarf virus in New York during the past four seasons.

The importance of using more than one aphid species to test plants for suspected infection by barley yellow dwarf virus (BYDV) was shown in comparative tests in 1959 with four aphid species (3). One purpose of the present paper is to report results of additional experiments made during the 1960 growing season with the same four vector species.

In earlier tests, a vector-specific strain of BYDV transmitted by Macrosiphum granarium was found to be common in New York (1, 3). A second purpose of this paper is to present data on the prevalence of this strain of virus in New York during each of four consecutive years.

MATERIALS AND METHODS

The four aphid species were the same as those used previously (3) but one species has been reidentified and a different clone of another species was used. The aphid species were as follows: 1) Rhopalosiphum padi (L.), the oat bird-cherry aphid (previously referred to as R. fitchii (Sand.), the apple grain aphid); 2) Macrosiphum granarium (Kirby), the English grain aphid; 3) Rhopalosiphum maidis (Fitch), the corn leaf aphid; and 4) Toxoptera graminum (Rond.), the greenbug. The clone of greenbug used in 1959 has been found to be a physiological strain that transmits BYDV very rarely, if at all (4); therefore, another strain of greenbug supplied in 1959 by D. C. Arny from Wisconsin was used. Virus-free stock colonies of all aphids were started weekly from newly emerged nymphs and maintained according to precautions described previously (2). Although all aphid colonies have remained virus free, some aphids from each colony used always were tested as controls.

Each sample consisted of a single oat leaf collected from a field plant believed to be infected by BYDV. Since only one leaf was collected from each plant, every test represents a different field plant. Each leaf was cut, usually longitudinally, into four sections. Each section, placed in a separate dish containing moistened filter paper, was infested with one of the four aphid species as described previously (2, 3). The leaf sections were incubated at 15° C for an acquisition feeding period of 24 to 48 hours. A comparable group of aphids from each colony was placed in a separate dish on healthy leaves to serve as a nonviruliferous control.

At the end of the acquisition feeding period, the dishes were taken to the greenhouse, where aphids from each dish were transferred to three seedlings of California Red oats (C.I. 1026), in groups of about 10 aphids per seedling. The test seedlings had been grown in steam-sterilized soil in 4-inch pots. Most of the aphids used were mature apterous females, but often other forms were included in each group. Test seedlings were caged by means of pot cages during a 3-day inoculation test feeding period; then all aphids were killed by fumigation with

[1]Cooperative investigation of Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and Cornell University Agricultural Experiment Station; supported in part by a PHS research grant, E-2540, from the National Institutes of Health.

[2]Plant Pathologist, Crops Research Division, Agricultural Research Service, United States Department of Agriculture, and Associate Professor, Cornell University. Grateful acknowledgment is made to Maureen Quinn for assistance.

Table 1. Recovery of barley yellow dwarf virus by R. padi (RP), M. granarium (MG), R. maidis (RM), and T. graminum (TG) from field samples of spring oats.

Pattern of transmission (+) or nontransmission (-) by aphid species				Number of samples for each New York county falling into indicated group					
RP	MG	RM	TG	Genesee	Ontario	Orange	Schuyler	Seneca	Tompkins
-	+	-	-	4	3	4	4	3	46
-	+	-	+	0	0	0	0	1	2
-	+	+	+	0	0	0	0	0	2
+	-	-	+	0	0	0	0	0	1
+	+	-	+	0	0	0	0	0	1
-	-	+	-	0	0	0	0	0	1
-	-	-	-	0	0	0	0	0	2

γ-1,2,3,4,5,6-hexachlorocyclohexane (lindane) in a closed chamber. Plants were next placed on a greenhouse bench under supplemental illumination and were observed at intervals for at least 4 weeks. The reactions of all three plants in each pot generally were the same, but results were considered positive if any one of the three plants developed symptoms of infection by BYDV.

RESULTS AND CONCLUSIONS

BYDV was recovered from 72 of the 74 spring oats (with symptoms of infection by BYDV) collected in six counties of New York (Table 1). Although most of the samples were from one county, results from other counties were in general agreement. The virus was transmitted only by M. granarium from 64 samples and by this species from an additional 6 samples from which one or more of the other aphid species also transmitted BYDV. Both R. padi and T. graminum transmitted virus from 1 sample, and only R. maidis transmitted the virus from another sample.

Tests also were made on a few samples of other small grains. BYDV was transmitted from 4 of 5 samples of winter oats; from 3 by M. granarium only and from 1 by R. maidis only. The virus was transmitted from all 3 of the samples of spring barley tested; from 2 by M. granarium only and from 1 by all vectors except R. padi. The virus also was recovered specifically by M. granarium from a single sample of winter wheat and from a single sample of Setaria sp. growing as a weed in an oat plot near Ithaca.

All test plants infested with nonviruliferous control aphids remained healthy.

These results provide additional evidence of the value of using more than one aphid species in tests to confirm diagnosis of suspected infection by BYDV. For instance, the virus was recovered by R. maidis from 2 samples (1 spring and 1 winter oat) from which neither M. granarium nor R. padi transmitted BYDV. Tests with such virus isolates have shown that they represent a vector-specific strain of BYDV that is transmitted by R. maidis but is not transmitted regularly by R. padi or M. granarium. Since two vector-specific strains had been identified previously (2, 5), at least three vector-specific strains of the virus are now known to occur in nature.

Direct comparisons of M. granarium and R. padi in the transmission of BYDV from field samples of oats collected in New York have been made during the past four seasons. Results of tests in each of the 4 years are in close agreement (Table 2) and are considered indicative of

Table 2. Transmission of barley yellow dwarf virus (BYDV) by R. padi (RP) and M. granarium (MG) from field samples of spring oats with symptoms of infection.

Year	Number of plants tested	Number of plants from which BYDV was transmitted by aphid indicated: RP only	MG only	Both RP and MG
1957	32	2	26	1
1958	48	0	41	5
1959	43	1	36	4
1960	74	1	64	1
Total	197	4	167	11

the relative prevalence of the two strains differentiated by these two vectors. BYDV was recovered specifically by M. granarium from 167 of the 197 spring oats tested during the 4 years. In 1957, 1958, 1959, and 1960, the percentages of samples from which the virus was recovered by M. granarium (and not by R. padi) were 81, 85, 84, and 86, respectively. The disease was much more widespread in 1960 than in the other 3 years.

Several lines of evidence together indicate that M. granarium was the most important vector of BYDV in New York during the 4 years studied. First, it was the most prevalent aphid species observed in oat fields; it usually was observed on spring oats a month or more before R. padi became common. (Since the most common aphid species is not necessarily the most important vector, this observation alone is not significant.) Second, the present data (Table 2) indicate that over 80% of the infections in the field were by the virus strain specifically transmitted by M. granarium. Third, when infection occurred in potted oat seedlings which were fed upon by M. granarium during field-exposure tests in 1960, the virus strain recovered was always the one specifically transmitted by M. granarium.

The close agreement of data on prevalence of the strain of BYDV specifically transmitted by M. granarium in each of the 4 years may not be of great importance since the relatively few samples tested were not collected over a wide area of the State every year. The results do suggest, however, that this virus strain is the most prevalent one in most of New York and that no major shift occurred in its relative prevalence during the 4-year period.

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INFLUENCE OF VARIOUS CARBON AND LIGHT SOURCES ON SOME CULTURAL CHARACTERS OF FUSARIUM LYCOPERSICI[1]

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Abstract

Fusarium lycopersici (Sacc.) Wr. was grown in Czapek's agar medium with 1, 3, 5, and 10% each of dextrose, lactose, sucrose, maltose, and glycerol and under different light and dark conditions. The fungus behavior in each concentration was studied on a 12-day period. Concentrations of sugar and different light conditions had profound influence on the cultural characters of the fungus.

INTRODUCTION

Fusarium lycopersici (Sacc.) Wr., the common tomato wilt pathogen, is capable of utilizing glucose, levulose, galactose, mannose, xylose, sucrose, maltose, soluble starch or inulin (1, 5). Brown (2) made extensive observations using species of Fusarium isolated from apple trees. He studied the effects of light as well as different concentrations of nutrients on the cultural characters of the fungus, and concluded that varying environments as well as nutrients have profound influence on the species of Fusarium which he used. Buxton (4), working with Fusarium oxysporum, reported pigment production of the fungus under different light and dark conditions. Zachariah, et al. (6) also found that fluctuating light and temperature had marked differences on the behavior of the species of Fusarium. The purpose of the present investigation was to determine the cultural characters of the fungus isolated from tomato plants which exhibited wilt, grown 1) on a medium containing different carbohydrate sources and levels and 2) under various light conditions.

MATERIALS AND METHODS

Petri dishes containing 20 mls of Czapek's agar medium with various carbohydrate sources and levels were inoculated with 1 cm-discs of the fungus culture grown on PDA and incubated at 20° C. Three replicates were used for each test. The amount and sources of carbon were 1, 3, 5 and 10% each of dextrose, lactose, sucrose, maltose, and glycerol. The fungus was grown under the following conditions: 1) ordinary daylight conditions, 2) continued darkness, 3) continued light, and 4) alternating 24 hours light and 24 hours darkness. A 600-foot candle power fluorescent light was used as the source of light in 3) and 4). Growth was recorded at every 24-hour period, and Brown's (3) linear growth was considered. Sporulation, pigmentation, zonation and staling were also recorded visually. Two controls were used: one with Czapek's agar nutrient only, the other with water and agar. Results are shown in Table 1.

DISCUSSION

Regardless of the source of carbon, good linear growth of the fungus occurred under conditions of normal daylight and darkness, as well as under continued darkness. However, the latter promoted a dense mycelial mat, while sporulation and pigmentation remained unaltered under this condition.

Growth was less in both continuous light and in 24-hour light and dark periods. This tendency differed in glycerol, where alternating light and dark periods showed a decline in the rate of growth (Table 1).

The pigment produced by the fungus varied with the different carbohydrate levels and sources. Darkness favored the formation of yellowish pigment, which increased in intensity with high concentrations of dextrose, lactose, sucrose, maltose and glycerol. Continued light promoted creamy yellow pigment in 1 and 3% of dextrose and lactose, while pink and yellow mycelial mats were noted in dextrose and lactose at 5 and 10%, respectively. In sucrose the pigment was pink and increased in intensity in high concentrations. When grown on maltose, the fungus produced purple pigment in 1 and 3% concentrations, whereas in 5 and 10% it exhibited a yellowish pigment. In glycerol there was little pigment produced in the medium and

[1]Deep appreciation is expressed to Dr. Nestor E. Caroselli, Professor of Botany and Plant Pathology, for his guidance.

Table 1. Average growth in centimeters of Fusarium lycopersici grown on various sources and levels of carbohydrates under conditions of ordinary daylight and darkness (R), continued darkness (D), continued light (L) and alternating periods of 24 hours darkness and 24 hours light (A) recorded after 48 hours of incubation.

Conc.	Dextrose				Lactose				Sucrose				Maltose				Glycerol			
	R	D	L	A	R	D	L	A	R	D	L	A	R	D	L	A	R	D	L	A
1%	2.2	2.4	2.0	2.0	2.0	1.7	1.9	2.2	2.4	2.5	2.3	2.3	2.7	2.9	2.8	2.8	2.3	2.3	2.1	2.2
	3.2	3.6	2.8	3.2	2.8	2.6	2.9	3.1	3.6	3.8	3.2	3.1	4.3	4.3	4.1	3.4	3.5	3.6	3.0	2.8
	4.2	4.8	3.9	4.7	4.0	3.9	3.5	4.0	4.7	5.2	4.5	4.4	6.0	5.9	5.6	4.4	4.4	4.9	3.8	3.6
	6.7	6.4	4.5	5.2	5.5	5.0	4.7	5.3	6.0	6.4	5.4	5.0	7.3	7.3	7.0	5.7	6.2	6.3	5.0	5.3
	7.5	7.7	5.4	6.3	7.0	6.2	6.0	6.3	7.5	7.4	6.4	6.0	com.	com.	8.1	7.1	7.1	7.6	6.4	6.4
	com.[a]	com.	6.4	7.1	7.3	7.8	7.5	7.5	8.3	8.4	7.5	7.1			com.	com.	8.1	8.8	7.3	7.8
			7.6	8.2	com.	com.	8.3	8.4	com.	com.	8.1	8.0					com.	com.	8.2	8.4
			com.	com.			com.	com.			com.	8.8							com.	8.8
												com.								com.
3%	2.6	2.1	2.2	2.0	2.1	2.3	2.1	1.9	2.4	2.5	2.5	2.3	2.7	2.8	2.6	2.9	2.3	2.0	2.1	2.3
	3.8	3.8	3.6	3.0	3.1	3.3	3.0	3.0	3.5	3.7	3.3	3.2	4.0	4.2	4.1	3.3	3.7	4.0	3.2	3.2
	5.6	4.9	4.3	4.3	4.4	4.6	4.2	3.8	4.7	5.0	4.6	4.0	5.6	5.8	5.4	4.3	5.1	5.3	4.3	4.5
	6.8	6.1	5.2	5.2	5.6	6.2	6.0	5.2	6.0	6.1	5.8	4.6	6.8	7.3	6.9	5.4	6.2	6.8	5.4	5.6
	7.5	7.5	6.1	6.1	6.7	7.5	7.6	6.3	7.3	7.3	7.3	5.6	8.3	com.	8.2	6.7	7.2	7.8	6.2	6.7
	com.	8.5	7.4	7.4	7.6	com.	com.	8.0	7.8	8.0	8.4	6.3	com.		com.	8.0	7.8	com.	7.0	7.3
		com.	8.6	8.6	com.			com.	8.8	com.	com.	7.5				com.	com.		8.0	8.2
			com.	com.					com.			8.6							8.4	8.4
												com.							com.	8.7
																				com.
5%	2.4	2.2	2.2	2.2	2.0	1.7	2.0	1.7	2.3	2.5	2.4	2.1	2.9	2.7	2.7	2.8	2.0	2.1	2.1	2.1
	3.4	3.3	3.3	3.2	2.8	3.0	3.0	2.7	3.4	3.7	3.5	3.1	4.0	4.1	4.1	3.3	3.5	3.6	3.3	3.1
	5.3	5.1	4.1	4.5	4.0	4.2	4.1	3.8	4.9	5.2	4.6	4.6	5.3	5.4	5.5	4.3	4.7	4.7	3.6	4.4
	8.0	6.9	5.0	5.5	5.3	6.2	5.8	5.0	6.0	6.6	5.8	5.8	6.4	6.8	8.2	5.6	5.8	6.0	5.5	5.3
	com.	8.0	6.0	6.6	6.4	7.3	7.0	6.2	7.5	7.9	7.3	6.3	7.6	8.6	com.	7.1	6.7	8.1	6.2	6.6
		com.	7.6	7.2	7.8	com.	8.0	7.5	8.2	8.9	8.4	7.3	com.	com.		8.2	7.4	com.	7.2	7.1
			com.	8.7	com.		com.	8.1	com.	com.	com.	8.2				com.	8.2		7.8	7.8
				com.				com.				com.					com.		8.3	8.5
																			8.5	8.8
																			com.	com.
10%	2.3	2.2	2.2	2.1	2.0	1.8	2.0	1.7	2.2	2.5	2.2	2.2	2.8	2.9	2.8	2.7	1.9	2.1	2.1	2.1
	3.3	3.0	3.5	2.6	2.8	3.2	2.8	3.3	3.2	3.8	3.2	3.3	3.8	3.9	4.0	3.9	3.0	3.4	2.9	2.8
	4.8	4.6	4.0	3.8	3.9	4.2	3.7	4.0	5.0	5.3	4.6	5.0	5.1	5.3	5.3	4.6	4.0	4.2	3.6	3.6
	6.0	6.0	5.0	5.1	5.7	5.4	5.4	6.3	6.3	6.8	6.1	6.3	6.1	6.4	6.5	6.1	4.3	5.2	4.2	4.3
	7.3	7.6	6.1	6.0	6.4	6.9	6.3	7.2	7.5	8.1	7.3	7.3	7.3	7.4	7.3	6.8	5.3	6.1	4.7	4.9
	com.	8.2	7.2	7.0	7.6	com.	7.2	8.4	8.2	com.	8.2	8.2	com.	com.	com.	8.6	6.1	6.9	5.2	5.5
		com.	8.0	com.	com.		com.	com.	com.		com.	com.				com.	6.8	7.8	5.8	6.2
			8.9														7.3	8.5	6.4	6.7
			com.														7.9	com.	6.6	7.3
																	8.4		7.4	7.8
																	com.		8.4	com.
																			com.	

[a]com. means Petri dish completed by the fungal mat.

the mycelial mat was noticeably light pink in color. The intensity of the pigment was not affected by concentrations, however.

Under conditions of alternating light and dark periods, pigmentation increased in intensity in high concentrations of dextrose, lactose, sucrose, maltose and glycerol, and in all tests the pigmentation was pink.

The aerial mycelium grown under alternate light and dark periods produced alternate bands of thick and thin growth corresponding to dark and light periods, respectively. The intensity of sporulation of the pathogen varied, and visual observation was employed to determine the intensity. Normal daylight and darkness enhanced sporulation in all concentrations of dextrose, with the highest in 1 and 3% concentrations. Sporulation was found to increase in high concentrations of lactose, but when sucrose and maltose were employed as the source of carbon spores were not detected regardless of concentrations.

Under conditions of continued darkness, no sporulation occurred in dextrose, but the intensity of sporulation increased in high concentrations of lactose. In media containing maltose and sucrose the fungus produced no spores. However in the former, a few sporodochia were noted. Sporulation was observed in the presence of all concentrations of glycerol.

Under continued light, 1% dextrose stimulated spore production. When the fungus was grown on media containing lactose, and subjected to light, it produced spores of approximately the same number in all concentrations. No spores were noted in the presence of sucrose and maltose, but glycerol favored the formation of spores and the intensity increased with high concentrations.

The fungus differed in its ability to produce spores in alternating light and dark periods. Small amounts of spores were observed in 1% concentrations of dextrose and sucrose, while there was no sporulation in other concentrations. Media containing lactose and glycerol promoted sporulation, but the occurrence of spores was not influenced by the concentration. In maltose, only the 10% concentration favored sporulation, which was very sparse.

CONCLUSION

Fusarium lycopersici is capable of utilizing various sources of carbohydrates. The color of pigment and the degree of sporulation varied not only with the source but also with the levels of carbohydrates added to a medium.

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A STEM AND BRANCH ROT OF SNAPDRAGON[1]

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A stem and branch rot of snapdragon (Antirrhinum majus) in a commercial greenhouse range in Georgia was brought to the writer's attention in the autumn of 1958. The disease was observed in greenhouse beds and in pots, but not in seed flats. It was first evident as water-soaked cankers which soon developed white centers with purplish borders. Cankers usually continued to develop until the stem was girdled. When the disease occurred on the stem near the soil it caused a rapid deterioration and sloughing of the outer stem tissue and death of the plant. A canker developing on a branch or upper part of the plant caused the death of that portion of the plant above the canker, but progress downward was usually stopped and the plant continued to grow and produce side shoots as if it had not been pinched at the point of attack. Some beds were a complete loss during the late summer and early fall. Losses in pots were often 50% or more. The disease was more evident during warm periods and largely disappeared with cooler weather.

Isolations from diseased material yielded a species of Phytophthora. The two types of symptoms observed were readily produced by inoculation of snapdragon plants with the isolates obtained and the fungus was reisolated. Infection was not produced by inoculation of a black-shank-susceptible tobacco variety, Virginia 21, or of a resistant variety, Cokers 156.

The fungus produced oogonia and oospores readily. Antheridia were predominantly amphigynous. Oogonia were 26-31 microns and oospores 19-26 microns. When mycelium grown on pea broth was washed and transferred to distilled water sporangia formed. Sporangia, which measured 25-45 x 20-40μ, produced zoospores readily in distilled water. Growth of mycelium was present on corn meal agar after 4 days at 35°C. These characters indicate that the fungus is Phytophthora parasitica Dast. (10).

No record of P. parasitica on snapdragons in the mainland of the United States has been found, but Tucker (11) stated that in 1932 he received from Mehrlich a culture labeled P. melongenae which had been isolated from snapdragons in Hawaii. Tucker (10, 11) considered this P. parasitica. Hopkins (6) reported this fungus causing collar rot and wilt of snapdragon in Southern Rhodesia. Bouriquet (3) reported it from Madagascar, and Shepherd (8) from Mauritius, on snapdragon.

Harris (5) reported P. cactorum (Leb. & Cohn) Schroet. causing girdling of snapdragons at the ground level and their wilting and death in California. Other reports of this fungus attacking snapdragons have been made in Beaumont and Staniland (2) in England and by Mes (7) in South Africa. Sundararaman and Ramakrishnan (9) described P. pini var. antirrhini on snapdragons in India. Tucker (10) considered this fungus synonymous with P. cactorum.

Other species of Phytophthora reported as causing a stem rot of snapdragons are P. cryptogea Pethyb. & Laff. from Victoria (4) and from Tasmania (1), and P. palmivora (Butl.) Butl. from Puerto Rico (11) and from India (9).

The disease in the greenhouse range observed largely disappeared with the advent of cooler weather. The ground beds, the potting soil, and pots in which diseased plants were grown had been sterilized by steam. However, from the appearance of weeds, from the behavior of the disease, and from comments of the operators, it was concluded that steaming was inadequate. The following year a better steam sterilization was carried out. Thereafter none of the disease was observed even though the soil source was the same.

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CONTROL OF PHYTOPHTHORA FRAGARIAE WITH SOIL FUNGICIDES

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Abstract

In field tests a mixture containing propargyl bromide, methyl bromide, and chloropicrin (Trizone) was effective in reducing to a low level red stele symptoms on strawberry roots. Sodium N-methyl dithiocarbamate (Vapam), a mixture of methyl bromide and chloropicrin (Dowfume MC-2), and chloropicrin (Picfume) were less effective but also reduced the severity of the disease. Nabam (Dithane D-14) provided some reduction in symptoms but did not exhibit marked eradicative or chemotherapeutic properties in this test. Thiram (Tersan) was ineffective.

INTRODUCTION

Chemical control of the strawberry red stele disease incited by Phytophthora fragariae Hickman has been studied by several workers. Stoddard (4, 5) reported that disodium ethylene bisdithiocarbamate (nabam) (Dithane D-14) acted as both a chemotherapeutant and a soil sterilant. He found that two applications of 2000 gallons/acre of 1.5% by volume of nabam before planting gave 100% control of red stele in the field. Wooley (6, 7) found a number of materials to be ineffective, including nabam (1380 pounds/acre), methyl bromide (430 pounds/acre), trichloronitromethane (chloropicrin 49 gallons/acre), 1,2-dichloropropane plus 1,3-dichloropropene (D-D Soil Fumigant 139 gallons/acre), and 1,3-chlorobromopropene (CBP-55 276 gallons/acre). All materials except methyl bromide were applied without subsequently covering the soil with plastic covers. Wooley (7) also noted that the fungus occurred to a depth of at least 20 inches in the soil. Jeffers (2) found that chloropicrin (31 gallons/acre) and methyl bromide (436 pounds/acre) gave 96 to 99% control in field tests in which plastic covers were used, and he suggested that higher dosages of these materials might eradicate red stele. D-D (46 gallons/acre) and CBP-55 (46 gallons/acre) gave good control in pot tests with plastic covers but not in the field without plastic covers (2). Sodium dimethyldithiocarbamate plus the sodium salt of 2-mercaptobenzothiazole (Vancide 51), zinc ethylene bisdithiocarbamate (zineb), and tetramethylthiuram disulfide (thiram), but not nabam, gave good to moderate control but were only tried in pot tests (2). Ferric dimethyl dithiocarbamate (ferbam), nabam, thiram, zineb, formaldehyde, and chloropicrin have been suggested in various States for commercial red stele control (3).

Field-plot fumigation tests with six materials (Table 1) for red stele control are reported in this paper.

Table 1. Chemicals used in red stele control tests, 1958-60.

Commercial material[a]	Manufacturer	Composition
Dithane D-14	Rohm & Haas Company	22% nabam
Tersan	E. I. duPont de Nemours & Company	75% thiram
Trizone	Dow Chemical Company	8% propargyl bromide 31% chloropicrin 61% methyl bromide
Vapam	Stauffer Chemical Company	32% sodium N-methyl dithiocarbamate
Dowfume MC-2	Dow Chemical Company	98% methyl bromide 2% chloropicrin
Picfume	Dow Chemical Company	99% chloropicrin

[a]The mention of specific products is made for technical accuracy and does not constitute a recommendation by the United States Department of Agriculture of any one brand.

[1]The help of Mr. K. K. Shiroishi in obtaining the data is gratefully acknowledged.

Table 2. Incidence of red stele in Dixieland and Pocahontas strawberry plants following postplanting treatments with various fungicides, Glenn Dale, Maryland.

Fungicide	Rate/acre	Red stele rating[a]	% plants infected
Dithane D-14 (nabam)	364 gallons (+24,200 gallons water)	5.6	60.0
Tersan (thiram)	1027 pounds (+24,200 gallons water)	3.9	80.0
Water	24,200 gallons	4.1	86.0
None	--	4.0	88.3
LSD 5%	--	1.3	15.9

[a]1 = most roots dead; 2 = some steles red to the plant crown; 3 = steles red 7/8 length of some roots; 4 = 3/4; 5 = 1/2; 6 = 1/4; 7 = 1/8; 8 = a few root tips with red steles and oospores present; 9 = a few root tips dead but no red steles or oospores; 10 = no infection.

MATERIALS AND METHODS

Postplanting Applications: A field at Glenn Dale, Maryland, in which strawberries had been grown for 4 years was used for the test. Strawberries (Pocahontas and Dixieland varieties, 10 rows of each) were found to be uniformly infected with red stele in the 30 x 60-foot experimental plot area. Nabam and thiram were applied (see Table 2 for rates) with a subirrigation gun (140 psi) in May 1958 to the soil around established plants. Injections were made to a 6-inch depth until the soil was saturated at 18- to 24-inch intervals in the rows. Five 30-foot single-row nabam plots were randomized with four water injection and seven untreated plots. Only 1 thiram plot was used. One week after treatment plants from nabam and water-injected rows were dug, and the nabam-treated plants were then subdivided into two lots, one in which roots were washed free of soil and the other in which roots were left unwashed. Five plants from each of the three lots were then potted in sterile soil with five healthy Blakemore plants and were held in a greenhouse until red stele symptoms appeared the following winter. Plants left in the field plots were dug, washed, and rated (Table 2, footnote a) for red stele in March 1959.

Preplanting Applications: The field used in 1958 for postplanting studies was used in May 1959 to evaluate preplanting applications of Trizone, Vapam, Picfume, and Dowfume MC-2 (see Tables 1 and 2 for percentage composition and rates). A hand-operated soil-fumigation liquid injector was used to apply the first three materials at 12-inch intervals at a depth of 6 inches, and methyl bromide was applied as a gas from commercial pressurized cans. All treatments were made in undisturbed rows of strawberry plants infected with red stele. All plots were covered with plastic covers for 48 hours after fumigation. Three plots (12.5 x 14.5 feet) were treated with each material, and three plots were left untreated. Raised beds (3 rows/plot) were prepared 14 days after fumigation, and Blakemore strawberry plants were planted. Replantings were made later wherever plants died. Incidence and severity of red stele were determined in the plots in April 1960 by using the same methods employed in the postplanting applications. In evaluation of chemicals those spots in treated rows that were level with the middles were omitted.

RESULTS

Postplanting Applications: Nabam significantly reduced the severity of red stele and the percentage of infected plants in the field 1 year after treatment, but 60% of the plants were infected (Table 2). Nabam killed about half of the plants and weakened most of the rest, but a sufficient number of plants recovered to fill in the rows with runners 1 year after treatment. Thiram was ineffective in reducing red stele severity or incidence but was not phytotoxic. *P. fragariae* infections developed with equal severity in the potted strawberries dug from nabam-treated and water-injected plots 1 week after treatment and in the healthy Blakemore plants potted with them. Washing the root systems of nabam-treated plants free of soil before potting had no effect on red stele incidence.

Preplanting Applications: All four materials tested reduced the severity of red stele and

Table 3. Incidence of red stele in Blakemore variety strawberry plants following preplanting treatments with various soil fungicides, Glenn Dale, Maryland.

Fungicide	Rate/acre	Red stele rating[a]	% plants infected
Trizone	51 gallons	9.7	9.3
Vapam	49 gallons	8.4	25.3
Dowfume MC-2	892 pounds	7.9	36.3
Picfume	78 gallons	7.5	49.3
None	--	3.1	94.0
LSD 5%	--	1.9	26.7

[a]See Table 2 footnote a.

the percentage of plants infected as compared with untreated plots (Table 3). Trizone gave the most control; 9.3% of the plants were slightly infected as contrasted with 94% of plants severely infected in untreated plots. Strawberries planted 14 days after chloropicrin treatment died, and plants set 1 month after chloropicrin treatment failed to produce many runners by fall and were dwarfed and slow growing the following spring. There was no evidence of phytotoxicity to strawberries planted in plots receiving the other treatments.

DISCUSSION

The results with nabam in the present test are more in agreement with those of Jeffers (2) and Wooley (7) than with those of Stoddard (4, 5). Nabam applied to well-established infected plants reduced red stele severity but not sufficiently to be of practical value. No chemotherapeutic activity was found since root systems of the infected plants treated with nabam, washed to eliminate external sources of inoculum, and then potted in sterile soil developed red stele in new roots. Healthy plants potted with them also became infected.

Trizone and Vapam greatly reduced red stele in strawberry plants when used as preplanting treatments. The red stele infection level following treatment with Vapam was greater than with Trizone, but the difference was not statistically significant. Red stele was present in a significantly larger number of plants in methyl bromide and chloropicrin than in Trizone-treated plots. Control levels with methyl bromide and chloropicrin were intermediate between those reported by Jeffers (2) and Wooley (7), both of whom used lower rates than used in the present test.

In 5 of the 16 Trizone- and Vapam-treated rows from which data were taken no plants had red stele; in most of the other 11 rows only an occasional plant had a few infected root tips. One Trizone and three Vapam-treated rows accounted for 60 and 80% of the infected plants, respectively, in plots receiving these two treatments. Because of the topography of the field, much of this infection likely developed through recontamination from adjacent untreated plots as a result of soil washing, but failure of the treatments to eradicate inoculum originally present in the plots cannot be ruled out. The plots were in a low area in a rather level field. Water drained into the hollow from all sides. Ditches and boards failed to prevent washing of infested soil into treated plots; and where plant beds were low, subject to run-off from infected plants, or silted in, red stele was likely to occur. There is a report (1) that planting of strawberries on high ridged beds of untreated soil in infested fields helps reduce red stele incidence.

Treated plant beds that were ridged high had the least red stele regardless of the chemical treatments used. Plants in all check plots were almost completely infected whether or not the plant beds were ridged. Since the problem of evaluating soil fungicides for red stele control is complicated by the apparent ease with which inoculum is moved in runoff water, it would seem desirable in field tests to place untreated check plots so that the danger of reinfestation of treated plots might be minimized. Additional field tests designed to eliminate such reinfestation are in progress.

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CROPS RESEARCH DIVISION, AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE

STUDIES ON TRANSMISSION OF TOBACCO RINGSPOT VIRUS ON SOYBEAN AND WEED SUSCEPTS

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Summary

Two new hosts, Abutilon theophrasti and Polygonum hydropiperoides, were found to be susceptible to tobacco ringspot virus (TRSV) through mechanical inoculation. The following common weeds showed no evidence of harboring the virus through natural infection: Ambrosia artemisiifolia, Abutilon theophrasti, Ipomoea hederacea, Polygonum hydropiperoides, Solanum carolinense, Taraxacum officinale, and Xanthium pensylvanicum. Negative results were obtained in attempts to transmit TRSV from infected to healthy soybean plants by means of whiteflies (Trialeurodes vaporariorum and T. abutilonea), flea beetles (Chaetocnema spp.), bean leaf beetle (Cerotoma trifurcata), the spotted cucumber beetle (Diabrotica undecimpunctata howardi), the two-spotted spider mite (Tetranychus telarius), and several unidentified aphids.

In 1956 the bud blight disease of soybeans, caused by tobacco ringspot virus (TRSV), was prevalent in southern Illinois. In 1957 soybean bud blight was present in this area, but was not so widespread as in the previous year. The spread of the disease from the borders into the center of a soybean field suggested the possibility of an insect vector. In one of the most seriously affected fields, periodic sweep collections were made to determine the numbers and kinds of insects present. A whitefly (Trialeurodes abutilonea) was by far the most abundant species in all the collections. In the literature we could find no record of whiteflies occurring on soybeans.

Insect Transmission Tests: Attempts were made to transmit TRSV with whiteflies (Trialeurodes abutilonea) and T. vaporariorum), flea beetles (Chaetocnema spp.), the bean leaf beetle (Cerotoma trifurcata), the spotted cucumber beetle (Diabrotica undecimpunctata howardi), the two-spotted spider mite (Tetranychus telarius) and two unidentified species of aphids. Colonies of field-collected insects and mites were allowed to feed on virus-infected soybean plants for up to 24 hours and then transferred to six healthy soybean plants, each plant receiving a minimum of 10 insects. No evidence of transfer of TRSV from diseased to healthy plants was found.

Weed Suscepts: A number of common weeds in and around the affected fields were tested as possible hosts of TRSV. A few of the suspected weed-hosts showed mottling of the leaves or other virus-like symptoms; however, most of them were free from symptoms and were tested only because of their proximity to infected fields. Among these weeds were common dandelion (Taraxacum officinale), horse-nettle (Solanum carolinense), cocklebur (Xanthium pensylvanicum), ivy-leaved morning-glory (Ipomoea hederacea), common ragweed (Ambrosia artemisiifolia), velvet-leaf (Abutilon theophrasti), and mild water-pepper (Polygonum hydropiperoides. Ten plants of each species were tested for virus content as follows: 10 leaves, one from each plant, were ground together in a phosphate buffer solution and inoculated on the primary leaves of 10 young soybean plants. TRSV was not recovered from any of these weeds. S. carolinense, I. hederacea, A. theophrasti, and P. hydropiperoides were grown in the greenhouse (75° to 80° F) and inoculated mechanically with TRSV from infected soybean plants.

The only suscepts of the virus in these tests were Abutilon theophrasti and Polygonum hydropiperoides. Systemic infection occurred in both species. Polygonum developed faint ringspots with no necrosis and with some leaf distortion. Abutilon showed a chlorotic mosaic mottling with some leaf distortion and occasional veinbanding. The virus was recovered from both species 2 months after inoculation. These two species have not previously been listed as suscepts of TRSV. Tuite (3) recently reported natural infection in Ambrosia artemisiifolia and Taraxacum officinale.

Temperature Effects: To determine the optimum temperature for symptom expression in Abutilon, plants in the 4- to 5-leaf-stage were inoculated with TRSV and incubated at 70°, 80°, and 90° F. Six inoculated and six uninoculated or control plants were held at each temperature and observed daily for expression of symptoms. This experiment was repeated twice.

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FIGURE 1. Leaf of Abutilon theophrasti at right showing systemic symptoms after inoculation with tobacco ringspot virus. Uninoculated plant at left.

The first symptoms appeared on the plants held at 80° and 90° F 5 to 8 days after inoculation. At 90° a faint chlorotic mottling was the most common symptom, but occasional leaves showed some veinbanding. At 80° the new leaves showed some distortion and prominent mottling after expansion. Raised islands of puckered leaf tissue frequently occurred in the mosaic pattern. At 70° the symptoms developed more slowly (8 to 11 days) than at the higher temperatures, but this was definitely the best temperature for symptom expression. The chlorotic mottling was very prominent (Fig. 1) and continued to appear on the new leaves for over 3 weeks. In contrast, symptoms persisted for only 6 days at 90° and for 9 days at 80°, after which the plants "recovered."

Seed Transmission: Approximately 300 seeds collected from infected Abutilon plants were germinated in pots of soil in the greenhouse to investigate the possibility of seed transmission. No recognizable symptoms developed on the resulting plants. Attempts to transmit TRSV in sap from these plants to soybeans gave negative results.

Whitefly Tests: Four attempts were made to transmit TRSV from soybeans to Abutilon and from Abutilon to Abutilon by means of a whitefly (Trialeurodes abutilonea). In one series, adult insects, taken from infected soybean plants, were caged on six healthy Abutilon seedlings (20 to 30 insects per plant) and allowed to feed for 24 hours. In another series, groups of 20 to 100 whiteflies were caged on infected Abutilon plants for 24 hours and then transferred to six healthy Abutilon seedlings, where they were left for 20 days. There was no evidence of transmission of TRSV from soybeans to Abutilon or from Abutilon to Abutilon by whiteflies.

DISCUSSION

The possibility that some of the weed species tested in these experiments may harbor TRSV still exists in spite of the negative results obtained. Limited numbers of each species were sampled and selected only at random; consequently, infected symptomless plants might have been missed. The finding of two of these species naturally infected in Indiana (3) further supports this possibility. In 1957 and 1958, when this work was done, bud blight was not extensive on soybeans and the incidence of infection may have been correspondingly low in weed suscepts. Abutilon might therefore be a carrier of the virus and as such would be of considerable interest since it is one of the most common annual weeds in Illinois soybean fields. If, as indicated by limited tests, the virus in Abutilon is not seed-borne, Abutilon would be of less epidemiological importance than a perennial weed host. In view of the facts that all attempts to demonstrate seed transmission of TRSV in soybeans were unsuccessful until 1954 (2) and

that the time of infection largely governs the degree of seed transmission (1), a more detailed study of seed transmission in Abutilon is needed.

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CONTROL OF CLADOSPORIUM SPOT OF SOUTHERN PEA

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Abstract

Eighteen varieties of southern pea were evaluated for resistance to Cladosporium spot. All were highly susceptible to the disease with the exception of Louisiana Purchase and Blue Goose, which were highly resistant. Of the several fungicides tested for control of this disease, maneb applied at bloom and at weekly intervals was more effective than the other materials, although none was adequate.

Cladosporium spot of southern pea, Vigna sinensis, incited by Cladosporium vignae Gardner, is the most serious disease of this crop in the mountain area of North Carolina. Entire fields which produce no marketable pods are encountered frequently; hence, the acreage planted to this crop has decreased markedly during the past several years.

Various varieties and breeding lines were evaluated for field resistance to the disease. (Breeding lines were obtained from A. P. Lorz of the University of Florida). Plantings consisted of three replications of 15-foot plots. Results are summarized in Table 1. All peas tested were very susceptible to the pathogen, with the exception of Louisiana Purchase which was essentially immune, and Blue Goose which was very resistant.

Table 1. Cladosporium spot disease index and severity of 18 varieties and breeding lines of southern pea. Figures presented are averages of three replications.

	Variety or breeding line	Disease index[a]	Severity[b]
1	73-05110	90	Slight
2	18-17-2110	100	Very severe
3	644210	88	Severe
4	Blue Goose	4	Slight
5	Running Acre	70	Severe
6	Lady Finger	100	Severe
7	Alabama Crowder	98	Severe
8	Cabbage Pea	98	Severe
9	Imported Bush Couch	99	Severe
10	California Blackeye No. 5	95	Severe
11	Purple Hull No. 49	78	Moderate
12	Louisiana Purchase	0	
13	Calhoun Crowder	100	Very severe
14	Brown Sugar Crowder	100	Slight
15	Early Ramshorn Blackeye	89	Severe
16	Big Brown Purple Hull Crowder	90	Severe
17	Black Crowder	89	Moderate
18	Lady or Rice	98	Very slight

[a]Disease index based on percentage pods with lesions.

[b]Severity based on size of lesions and degree of pod malformation.

In addition, several fungicides were evaluated on the susceptible variety Early Ramshorn Blackeye. Fifteen-foot plots were replicated four times with buffer rows between. The following fungicides were applied at bloom and at weekly intervals and after heavy rains: zinc ethylene bisdithiocarbamate (zineb 1 1/2 pounds/100 gallons), manganese ethylene bisdithiocarbamate (maneb 1 1/2 pounds/100 gallons), basic copper sulfate (Tri-Basic 4 pounds/100 gallons), and 2,4-dichloro-6-(o-chloroanilino)-s-triazine (Dyrene 1 1/2 pounds/100 gallons). A total of six applications were made with 3-gallon compression sprayers. The average number of pods infected of 200 pods observed per replicate was: zineb, 86; Tri-Basic, 114; maneb, 28; Dyrene, 48; and the unsprayed control, 172. Control with maneb was not adequate, but was superior to the other fungicides tested. Although the control of Cladosporium spot of pea by fungicides might be improved by increasing application frequency and number, such a spray program with southern pea would seem economically impractical. It is therefore suggested that Louisiana Purchase or Blue Goose be planted in areas where Cladosporium spot is a problem.

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CULTURAL VARIABILITY OF SELECTED ISOLATES OF RHIZOCTONIA SOLANI AND THIELAVIOPSIS BASICOLA, AND THE VARIABILITY IN THEIR PATHOGENICITY TO ACALA AND PIMA COTTON, RESPECTIVELY[1]

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Summary

Laboratory evaluation of 12 Rhizoctonia solani isolates selected from over 110 as "type" cultures yielded six different cultural type or "strain" reactions. The variability of these isolates in pathogenicity to Acala cotton ranged from non-pathogenic to highly-pathogenic, constituting four distinct classes as determined in greenhouse tests. These "strain" patterns were maintained through successive tests. Similarly, 11 isolates of Thielaviopsis basicola selected from over 120 in culture possessed considerable variability on five different media. Six "strain" or group reaction patterns were obtained by combining cultural variability with variability in pathogenicity to Pima cotton seedlings determined in the greenhouse. The variation shown by T. basicola isolates was considerably less than that shown by R. solani isolates.

Rhizoctonia solani Kuehn and Thielaviopsis basicola (Berk. & Br.) Ferr. cause the greater amount of damage due to seedling diseases of cotton in New Mexico, where they are included in a complex also involving Fusarium spp. and possibly other soil-borne fungi. R. solani damage to seedlings has been variously referred to as sore-shin, damping-off, and seedling blight; damage due to T. basicola is called black root-rot. In order to gain a better understanding of the variability of these two pathogens, as well as to establish a scientific basis for control measures, the present variability studies were undertaken.

The lack of specialization in R. solani and its range of pathogenicity to cotton has been the subject of much research, which has consistently led to the conclusion that this soil-inhabiting pathogen was highly variable and could incite disease in a wide variety of hosts (3, 4, 6). An earlier study by the writers disclosed ten distinct "strain" reactions on the basis of cultural and pathogenic variability of 245 R. solani isolates from cotton in New Mexico, and led to the present study (6).

T. basicola has been recognized as the causal agent in black root-rot of tobacco, and has recently been implicated in the seedling disease complex on cotton in New Mexico (5). Thielaviopsis basicola is more severe on Pima cotton than is Rhizoctonia solani, but is less damaging to Acala varieties. It causes a cortical rot of the primary root, and destroys many of the smaller roots of Acala seedlings, but causes a more severe rotting and subsequent killing of Pima (1). Studies have shown that Acala exhibits a high degree of recovery from attack by T. basicola.

RHIZOCTONIA SOLANI ISOLATES

Selection and Source: From over 110 R. solani isolates recovered from rhizosphere soil samples and infected cotton and bean plants, 12 were selected for inclusion in the present study. These 12 isolates represented "type" cultures on potato-carrot-dextrose agar (PCDA). The 12 isolates were obtained from the following areas: 2, Deming; 2, Animas Valley; 2, Portales; 4, Las Cruces; 1, Carlsbad, and 1, Artesia. Six each were recovered by plating infected plant sections and rhizosphere soil.

Variation in Culture on Various Media: Following the methods employed in the previous study (6), the isolates were transferred to three plates each of the four different media: potato-dextrose agar (PDA), carrot-decoction agar (CA), beef-peptone agar (BPA), and potato-carrot-dextrose agar (PCDA). The isolates grew at a uniform rate, but differed markedly in mycelial formation and reproductive capacity on the media employed (Table 1).

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Table 1. Cultural differences of 12 selected Rhizoctonia solani isolates on four different media.

	PDA	CA	BPA	PCDA
Cultural Characteristics				
1b	pr, aer & sub	mostly sub	aer & sub	pr, aer & sub
2c	pros, aer & sub	mostly sub	aer, fl	pros, aer & sub
3c	aer & sub	sub	aer & sub	mostly sub
4d	mostly sub	sub	aer & sub, ap	aer & sub
5a	aer & sub	sub, sp	mostly sub	mostly sub
6c	aer & sub	mostly sub, ap	mostly sub	sub
7a	aer & sub	sub	mostly sub	aer & sub, ap
8f	mostly aer, pros	sub	aer & sub	aer & sub, fl
9c	aer & sub	sub	mostly sub	aer & sub
10d	mostly sub	mostly sub	aer & sub	mostly sub
11c	mostly sub	sub	sub	mostly sub
12b	mostly aer	aer & sub	aer & sub	aer & sub, fl
Sclerotial Characteristics				
1b	none	mod, aer	mod, sub	sp, aer
2c	none	sp, sub	pr, aer & sub, zon	sp, aer
3c	pr, mostly aer	sp to la, sub	sp, sub	mod, mostly sub
4d	mod-sub	none	mod, aer & sub	sp, sub
5a	sp-sub	none	none	none
6c	none	none	mod, mostly sub	none
7a	none	none	pr, aer & sub, zon	sp, sub
8f	pr, sub	none	sp to la, aer	none
9c	pr, sub	none	mod, aer & sub	pr, mostly sub
10d	none	none	sp, mostly sub	none
11c	pr, sub, zon	sp to la, sub	sp, mostly aer	pr, sub
12b	mod, aer	none	mod, aer & sub	mod, mostly aer

Key: pr = profuse; aer = aerial; sub = submergent; pros = prostrate; ap = appressed; sp = sparse; fl = fluffing; la = lacking; mod = moderate; zon = zonate.

Table 2. Pathogenicity of 12 selected Rhizoctonia solani isolates to American Upland cotton seedlings, as expressed by a disease severity index.

Isolate No.	Disease severity index[a] Trial 1	Trial 2	Mean	Pathogenicity rating[b]
1b	6.2	7.7	6.95	moderate
2c	3.4	4.2	3.80	slight
3c	9.5	8.4	8.95	high
4d	2.1	2.5	2.30	slight
5a	6.5	5.6	6.05	moderate
6c	8.1	9.2	8.65	high
7a	9.8	9.5	9.65	high
8f	8.8	8.6	8.70	high
9c	1.2	1.3	1.25	non-path.
10d	2.5	2.6	2.55	slight
11c	6.6	6.4	6.50	moderate
12b	9.5	8.4	8.95	high
Check	0.8	1.1	0.95	--
Difference (.01) for significance	1.15	1.23	--	--

[a]Disease severity index calculated on a 0-10 scale from seedling survival and root-rot or lesion severity of surviving plants. Each trial, mean of four replicates of 50 plants each.

[b]Pathogenicity rating based on the Duncan MR test analysis.

Good mycelial development occurred on PDA; four isolates developed mostly submergent mycelia, two mostly aerial, and the remaining six produced both types. Growth was somewhat slower on CA, as all isolates except 12b produced submergent or closely appressed mycelia. More profuse growth was made on BPA, and five isolates developed submergent or closely appressed mycelia, six both aerial and submergent mycelia, and only 2c produced some fluffy aerial hyphae. The greatest variation in growth was shown by the isolates on PCDA; five isolates produced mostly submergent mycelia, five closely appressed aerial mycelia, and two both types, with isolate 8f developing some fluffy hyphae.

Reproductive body formation varied greatly on PDA, as five isolates produced few to no sclerotia, two mostly aerial sclerotia, and five submerged sclerotia. Sclerotia on this medium differed little in size or color; the most profuse aerial formation was shown by isolate 3c. Sclerotial formation on CA was generally sparse. Eight isolates produced no sclerotia, three produced only a few submerged sclerotia and 1b a moderate amount of aerial sclerotia. Reproductive capacity of the isolate was greatest on BPA, as only isolate 5a produced no sclerotia, four produced mostly submerged sclerotia, two mostly aerial sclerotia, and five both aerial and submerged sclerotia. Sclerotial color varied from light tan to deep red-brown. On PCDA, four isolates produced no sclerotia, three mostly aerial, three only submerged sclerotia, and two both aerial and submerged sclerotia.

Sclerotial production was sparse to fair for all isolates except 9c and 11c, which produced moderate to profuse sclerotia on all media except CA. Considerable variation occurred in the patterns of sclerotial formation (zonation) among the isolates, especially on PCDA. The above variation in the 12 R. solani isolates appears to fall within the variation range reported in the previous study, representing six type (or "strain") cultural patterns.

Pathogenic Variability: Pathogenic variability of the 12 R. solani isolates was tested on variety 1517C cotton seedlings in two successive trials, using four replicates of three pots, each containing 10 seedlings. Soil was steam-sterilized, placed in 6-inch pots, and heavily infested with an oat-infusion of each R. solani isolate; one replicate of non-infested soil was included. After 3 days' incubation, 10 germinable seeds per pot were planted. After 3 weeks the plants were carefully removed, rated for severity of R. solani damage, and a disease severity index calculated for each replicate. A scale of 0-10 was employed, 0 being no infection, to 10 being complete loss of all seedlings. The pathogenicity indices of the isolates (Table 2) fall into four categories, based on the Duncan MR test analysis, which were designated highly, moderately, slightly, and non-pathogenic. The non-infested check was the basis for rating pathogenicity, with its disease index apparently due to reinfestation of the sterilized soil by R. solani or other disease organisms. Five isolates were highly pathogenic, three moderately pathogenic, three slightly pathogenic, and one (9c) non-pathogenic.

Variability Grouping on Cultural and Pathogenic Variation: From the variability as shown by the measured parameters, cultural type characteristics and pathogenicity, the 12 R. solani isolates appear to comprise 10 variability or "strain" groupings. This is consistent with the previous study (6), with the variability groupings closely akin to those reported. The only apparent conclusion is that R. solani is a highly variable species, but the individual isolates are consistent in their variability, one from another. These isolates will be further tested for variability in response to treatment with a number of fungicidal chemicals incorporated into the media.

THIELAVIOPSIS BASICOLA ISOLATES

Selection and Source of Isolates: In the same manner, 12 isolates of T. basicola were selected from 120 recovered as representing cultural "types" on potato-dextrose agar. The T. basicola isolates were obtained chiefly from the Luna-Hidalgo County areas (2, Lewis Flat; 3, Animas Valley; and 3, Deming), with 2 from the Mesilla Valley and 1 each from Portales and Carlsbad. The Portales isolate was accidentally lost after the study commenced, leaving 11 in the evaluations. All isolates were recovered from diseased cotton seedlings by plate isolation on PDA, and were of the gray type described by Stover (7).

Cultural Variation on Different Media: The 11 isolates of T. basicola were tested for cultural variability in the same manner described for R. solani. Five media were employed, however: Martin's soil medium (MSM), PCDA, peptone-dextrose agar (PeDA), Oxgall agar, and PDA. The variation in rate of growth among the isolates on the different media was striking, ranging from almost nil to excellent (Table 3). PDA was generally the most favorable medium for the isolates, but less variation in growth occurred on PCDA. Growth rate of the isolates was recorded by a 0-5 scale, where 0 = no growth from original planting, and 5 =

Table 3. Cultural variation of 11 selected Thielaviopsis basicola isolates on five different media.

Isolate No.	MSM			PCDA			PeDA		
	Growth rate[a]	Mycelial texture	Chlamy. for.[b]	Growth rate	Mycelial texture	Chlamy. for.	Growth rate	Mycelial texture	Chlamy. for.
9E1	3	sp, ap	2	4	mod, ap, aer	2	2	sp, sub	-
18F1	2	mod, ap	2	3	mod, sub	2	2	sp, ap	1
26A1	2	sp, ap	1	2	mod, ap	3	2	sp, sub	2
24A2	1	sp	1	4	ex, ap	2	3	mod, sub	-
24F1	2	sp, less ap	2	3	mod, ap	1	2	sp, ap	1
27F1	3	mod, ap	2	5	ex, less ap	2	3	mod, ap	-
27B2	4	mod, sub	2	4	ex, ap	1	3	mod, less ap	-
5B1	2	sp, ap	2	3	sp, ap	3	2	sp, aer	2
15A2	2	sp, ap	2	3	mod, ap	3	1	sp, ap	1
10C1	1	sp to la	3	2	sp, ap	3	1	sp, sub	-
3E1	1	sp, ap	2	3	ex, aer	1	1	sp to la, sub	-
	OXGALL A			PDA			Mean, all Media		
9E1	1	la, ap	-	2	sp, ap	1	2.4		1.0
18F1	1	sp, ap	1	5	ex, less ap	3	2.6		1.8
26A1	1	sp, sub	-	4	ex, ap	3	2.2		1.8
24A2	1	sp, ap	-	4	ex, ap, zon	2	2.4		1.0
24F1	1	sp, ap	-	3	mod, ap, thin	1	2.4		1.0
24F1	2	mod, less ap	2	3	mod, ap	2	3.2		1.6
27B2	1	sp to la	1	4	ex, sub, zon	2	3.2		1.2
5B1	1	sp, sub	1	4	ex, less ap	3	2.4		1.8
15A2	1	sp, ap	-	3	mod, ap, ragged	2	2.0		1.6
10C1	1	sp, ap	-	2	sp, aer, sec	3	1.4		2.0
3E1	1	sp, sub	-	5	ex, aer & sub, zon	3	2.2		1.2

[a]Growth rate scale 0-5: 0 = none from planting to 5 = plate covered in 14 days.
[b]Chlamydospore formation scale 0-3: - = nil; 1 = sparse; 2 = moderate; 3 = profuse.
Key: sp = sparse; mod = moderate; ex = extensive; ap = appressed; aer = aerial; sub = submergent; la = lacking; sec = sectoring; zon = zonate.

plate covered in 14 days. Best growth over the range of media was shown by isolates 27B2 and 27F1, and poorest by 10C1. The isolates showing best growth on each medium were: 27B2 on MSM; 27F1 on PCDA; 24A2, 27F1, and 27B2 on PeDA; 27F1 on Oxgall agar; and 3E1 and 18F1 on PDA (Fig. 1).

Variation in mycelial texture and growth habit was less than that of growth rate. The appressed habit with sparse mycelial development appears the norm for T. basicola, with little or no submerged growth and chlamydospore formation at the medium surface. One or two isolates exhibited limited submerged hyphal development on each medium, however. Chlamydospore formation ranged from nil to profuse on the various media. Isolate 10C1 produced chlamydospores most abundantly on the various media, followed by 26A1 and 5B1. Poorest chlamydospore production occurred in isolates 9E1 and 24F1. All isolates produced fair to profuse endoconidia. Additional isolates in culture, and others subsequently isolated, appear to fall

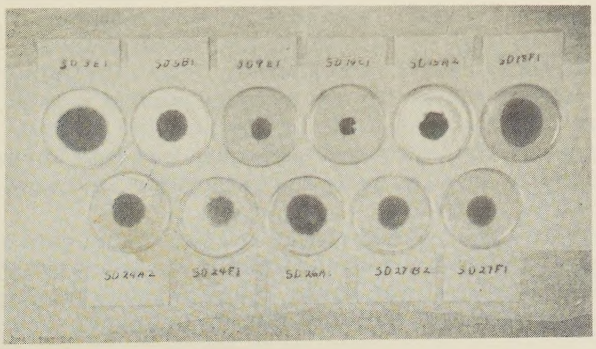

FIGURE 1. Selected T. basicola isolates on PDA, after 8 days' growth at 26° C, demonstrating the observed cultural variation.

Table 4. Pathogenicity of 11 selected Thielaviopsis basicola isolates to American Egyptian cotton seedlings, based on a disease severity index, from the second of two trials.

Isolate No.	Black root-rot severity index[a]	Pathogenicity rating[b]
9E1	63.4	moderate
18F1	55.3	slight
26A1	71.9	moderate
24A2	65.6	moderate
24F1	76.1	high
27F1	77.7	high
27B2	86.6	high
5B1	84.1	high
15A2	53.0	slight
10C1	35.2	non-path.
3E1	94.5	high
Check	18.2	--
Difference (.01) for significance	19.72	

[a]Disease severity index calculated from plant survival and black root-rot severity of surviving seedlings on a 0-100 scale. Indices are the means of four replicates of 20 plants each. Trial I indices were in close agreement.

[b]Pathogenicity rating based on Duncan MR test analysis. 94.5-75.6, high; 75.5-60.3, moderate; 60.2-35.8, slight; and 35.7 or less, non-pathogenic.

Table 5. Distribution of 11 selected Thielaviopsis basicola isolates according to "type" reaction based on cultural and pathogenic variability.

Type reaction	Variation types shown[a]			Isolate No.
	growth	sporulation	pathogen	
I	+++	+++	+++	27B2, 5B1, 27F1, 24F1
II	+	+	+++	3E1
III	+++	++	+	18F1
IV	+	+	++	9E1, 24A2
V	+	++	+ (++)	26A1, 15A2
VI	+	+++	-	10C1

[a]Number of plusses in column and at each heading indicates rates of growth, amount of sporulation, and pathogenicity rating.

within the range of cultural variation shown by the test isolates. Six different type reactions were distinguished on the basis of growth rate, mycelial development, and sporulation.

Pathogenic Variability to Pima: A wide range of pathogenicity to Pima 32 seedlings was exhibited by the 11 isolates of T. basicola. Two successive trials were conducted in the greenhouse, using the procedures described for R. solani with the exception that small flats, each holding 20 seedlings, were used instead of clay pots. Disease severity and seedling survival were recorded after 14 days, and the disease severity index calculated for each replicate (Table 4), which reflects the damage due to T. basicola relative to the non-infested check flats. The two successive trials showed close agreement in pathogenicity. The 11 Thielaviopsis basicola isolates occupied four classes via the Duncan MR test analysis applied to trial 2. Five isolates were designated highly pathogenic, three moderately pathogenic, two slightly pathogenic, and one non-pathogenic. The range of variability was somewhat less than for the R. solani isolates, with only one of the T. basicola isolates being non-pathogenic, and none of the highly pathogenic ones showing total kill of the seedlings.

Variability Grouping: Based on both cultural type characteristics and pathogenic capability on Pima 32 cotton, the 11 Thielaviopsis basicola isolates were classified in six group or "type" reactions (Table 5): I. good growth, good chlamydospore production, and high pathogenicity; II. relatively poor growth, poor sporulation, and high pathogenicity; III. rapid growth, moder-

ate reproduction, and low pathogenicity; IV. poor growth and sporulation, but moderate pathogenicity; V. poor growth, moderate sporulation, and low to moderate pathogenicity; and VI. poor growth, good sporulation, and a low order of pathogenicity (non-pathogenic). The variation obtained for the T. basicola isolates employed in the current study appeared to be somewhat greater than that obtained by Johnson and Valleau (2), but less than that described by Stover (7).

DISCUSSION

The variability which can be demonstrated for R. solani isolates would perhaps be even greater than that demonstrated on the basis of cultural characteristics and pathogenicity to Acala cotton had other parameters been employed, such as host range, types of symptoms produced on certain hosts, methods of survival and disease development, or growth on certain organic soil amendments. From the present study it appears that while R. solani isolates are almost universally present in New Mexico cotton soils, those present in some soils cause far more serious damage than those present in other soils. The same holds true for isolates of T. basicola; isolates recovered from the Mesilla Valley have shown a moderate degree of virulence, while those from other areas have shown a generally lower level of pathogenicity. How these demonstrated variations might be applied to effect control is the subject of further study.

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BOOK REVIEW

"PLANT DISEASE HANDBOOK" (Revised Second Edition), by Cynthia Westcott. D. Van Nostrand Company, Inc., Princeton, N. J. xii +825 pages. 1960. Price $13.50.

If you've been looking for a helpful book that will guide you in protecting ornamental plants from the destroying wilts, blights, and rots, look no further. Whether you are an amateur or a pro in the gardening business, Cynthia Westcott's new handbook can be very useful.

This new, revised edition of "Plant Disease Handbook" is an up-to-date version of Dr. Westcott's original handbook, issued in 1950 -- a volume that has stood the test of time very well. The new handbook contains information on diseases that attack the ornamental plants of the United States; where they occur; what they look like; and what to do about them. Comparing this edition with the first handbook, the reader becomes aware of just how far the science of plant pathology has advanced in 10 years. This volume contains much new information on nematodes and virus diseases and on chemical control.

Dr. Westcott is the original "plant doctor." She is one of the few -- and perhaps the first -- university-trained plant pathologist to seek a career serving the practical needs of the individual nurseryman, landscape specialist, and gardener. And this book reflects her career. It contains information pertinent to the gardener with a disease problem. It is organized to be immediately useful. It is understandable.

Once an interested reader has skimmed through the 800 pages of this handbook, it is likely that chapter five will serve as his point of reference for further use. Here, in some 300 pages, Dr. Westcott has alphabetically listed more than a thousand flowers, trees, shrubs, and lawn grasses of the United States and listed the diseases that attack each and the States where each disease is known to occur. With this information, the reader can dig into other chapters where the disease is described (and in many instances illustrated) and effective treatments are prescribed.

Dr. Westcott also includes in her handbook an introduction that helps to put plant diseases and their control into perspective; a glossary that translates the language of plant pathology into every-day English; and a selective index that includes the common and Latin names of ornamental plants, common names of diseases which attack them, Latin names of disease pathogens, and the chemicals used to control them.

Diseases and pathogens are grouped in a chapter under the headings by which they are commonly known -- anthracnose, bacterial diseases, blackleg, and so on, to the wilts. Dr. Westcott's chapter on chemicals and treatments includes not only a complete listing and description of present-day materials, but information on mixing and applying these chemicals safely and effectively. -- PAUL R. MILLER

CORRECTION

REPORTER, July issue (Volume 44, Number 7). Dr. T. van der Zwet has called to our attention the omission of a very important part of a sentence in the article on pages 519-523.

On page 519, third paragraph, the third sentence, beginning on line 4 should read "When these Phytophthora strains were examined, one was identified as P. erythroseptica Pethyb., one was found to resemble Pythium in its morphological ... etc."